YELLOW FEVER

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YELLOW FEVER

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PREFACE

THE PART played by the International Health Division of The Rockefeller Foundation in the world wide campaign against yellow fever, which covered a period of more than thirty years, represents an experience that can be studied with profit by epidemiologists, virologists, entomologists, biologists, mammalogists climatologists, and ecologists, as well as practically everyone interested in a subject involving any of the biologic sciences The story, moreover, has intrinsic interest

The work was begun at a time when there was real danger that the disease might spread to countries of dense population, such as India It never did spread to India, although one of the well known mosquitoes that carries the disease is found in abundance in that country The story of the conquest of yellow fever is full of unexpected turns, unlooked for puzzles and unanticipated and exasperating set backs, as well as triumphs with steady progress achieved only as the result of firmly sustained and many sided effort

The comparatively vast scientific effort expended in laboratory and field work had its moments of sadness and drama During the campaign six scientists on active duty succumbed to the disease Howard B Cross contracted fatal yellow fever while on a field assignment in the State of Veracruz, Mexico Four scientists collaborating with the West Africa Yellow Fever Commission died of laboratory infections Adrian Stokes Hideyo Noguchi William Alexander Young, and Theodore B Hayne At the Yellow Fever Laboratory of the International Health Division in Bahia, Brazil, Paul A Lewis contracted a fatal infection To these pioneers who, in advancing yellow fever research, made the supreme sacrifice of life itself, we pay tribute

One of the incidental benefits of yellow fever research was that it set a pattern for similar studies of other diseases No reader of the chapters that follow will fail to note that the varied techniques here described are of wide application In connection with one phase of the yellow fever work in Brazil, some 400,000 liver specimens were collected and made available for study As a consequence of their examination, two diseases new to Brazil were identified, and much information about the distribution and intensity of two other diseases was secured At other times the work led into elaborate ecologic studies of animals in their native habitats Incidental to the search for the virus of yellow fever, 16 new viruses were isolated, thus pointing to

the tropics as a likely primary reservoir of viruses from which on occasion they escape to the temperate zones The whole effort might be considered an overture to further study of the much wider subject of virus diseases in general

In portraying the activities of the International Health Division of The Rockefeller Foundation special credit is due Mr Wickliffe Rose who as Director of the earlier International Health Commission inaugurated the studies on yellow fever and Dr F F Russell his successor who provided inspiration and personal direction necessary for its successful development Similar recognition should be accorded to Dr Wilbur A Sawyer who took up where Dr Russell left off and was responsible for many fundamental advances in both laboratory and administrative techniques However it must be emphasized that the contribution of the Division was the joint product of dozens of staff members scores of other scientists and an even greater number of native born participants in the countries of Latin America and Africa The role of the anonymous members of this army of workers was no less important than that of those whose publications have been specifically referred to in this book and in the accomplishments reported credit goes alike to each and all of these men

During the years the campaign was in operation 122 yellow fever articles largely scientific in character were published by the staff members of the International Health Division and their collaborators Although these articles are available in many libraries it was considered desirable and useful to review the entire enterprise within the confines of a single volume and to this end Yellow Fever is presented Free use has been made of the annually published reports of the International Health Division containing material that is in turn based on contributions as well as field reports from a wide range of former staff members many of them eminent authorities on yellow fever The selection of authors was dictated by the following considerations all had worked in the Foundation s yellow fever program all had made noteworthy contributions to the subject and finally all were staff members currently stationed in New York and able to be in constant contact with one another during the publication schedule

This book makes no claim to be encyclopedic It does not attempt to provide a complete bibliography All the articles by those who participated in the yellow fever program are listed and usually discussed but the work of other investigators is mentioned only when pertinent to the discussion.

Each chapter is a fairly complete treatise on the subject with which it deals Since the subjects necessarily overlap there is a certain degree of duplication in the volume

The editor in chief and the several authors desire to express deep appreciation to those who aided in the preparation of the book First we wish to thank the Trustees of The Rockefeller Foundation and Mr Chester I Barnard its president as well as the Board of Scientific Consultants of the International Health Division for their encouragement and material support in appropriating funds so essential to the enterprise To the Office of Publications of The Rockefeller Foundation under the leadership of Mr H B van Wesep we are indebted for the painstaking task of editing the manuscripts and checking references

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January 1951

1 LANDMARKS IN THE CONQUEST OF YELLOW FEVER

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In all of ancient medical literature there is no reference to yellow fever by that or any other name Medical writing of early times abounds with descriptions of diseases that we now know by names other than those assigned by the writers but nowhere has there been found an account of a malady identifiable as yellow fever Since epidemic yellow fever is a dramatic disease it seems unlikely that it could have escaped the attention of even very early medical writers It is perhaps reasonable to conclude that the disease did not exist in ancient civilizations

It was not until the seventeenth century that yellow fever was recognized as a disease entity First identified in America it has sometimes been referred to as a disease of the New World The exact place of origin of yellow fever has been subject to much controversy There is considerable agreement however that the disease originated either in Africa or in tropical America that is in areas in which the disease is today endemic Both these regions were opened to European communication at practically the same time After the circumnavigation of Cape Verde in 1444 and a landing on the Guinea Coast in 1471 a first settlement was made there in 1482 Communication between tropical America and West Africa began early and continued regularly with ample opportunity for the two areas to exchange their respective infectious diseases

Most early history of diseases and epidemics particularly in the New World was recorded by laymen Not a few of the epidemics in the New World since 1492 have been attributed by these lay medical historians to yellow fever But it is certain that many of them could not have been this disease and were probably plague smallpox cholera malaria typhus or other maladies A detailed account of the early history of yellow fever may be found in the work of Dr Henry Rose Carter (1931) an eminent epidemiologist who devoted the last years of his life to a study of the origin of the disease According to Garrison (1929) the term yellow fever was first employed by Griffin Hughes in his Natural History of Barbados (1750)

Urban yellow fever—the classic epidemic type—is due to a filtrable virus and is transmitted from man to man by the mosquito *Aedes aegypti* Once the mosquito becomes infected the infection is retained for the rest of its life *A aegypti* an essentially domestic species which breeds by preference

in artificial water containers, could, by haunting the water casks of sailing ships, distribute yellow fever infection to places a long distance from endemic or epidemic foci And indeed it was by ship that the disease reached New York in 1668, Boston in 1691, and Charleston, South Carolina, in 1699 It did not reach Europe until the following century

For more than two hundred years yellow fever was one of the great plagues of the world The tropical and subtropical regions of America were subject to devastating epidemics, while serious outbreaks occurred as far north as Boston and as far away from the endemic centers as Spain, France, England, and Italy During this period appalling epidemics swept repeatedly over the West Indies, Central America, and the southern United States, decimating populations, paralyzing industry and trade, and holding the people of these regions in a state of perpetual dread It is estimated that the epidemic which visited the Mississippi Valley in 1878 caused 13,000 deaths and by bringing business to a standstill resulted in an economic loss of more than one hundred million dollars Each succeeding summer brought with it the possibility of a repetition of the experience Philadelphia suffered 20 epidemics, New York 15, Boston 8, and Baltimore 7. As late as 1905 New Orleans was invaded, as were other ports of the South, there were 5 000 cases and 1,000 deaths

EARLY RESEARCH AND CONTROL

The economic consequences of an epidemic of yellow fever, resulting from the actual number stricken and intensified by the fear and dread of the disease, gave rise to a complete interruption of normal intercourse, both business and social The atmosphere of extreme apprehension was due to ignorance regarding the cause, means of transmission, and prevention of yellow fever, and to the fact that the disease was generally regarded as contagious This state of ignorance in turn led to all sorts of wild speculation and theorizing

What turned out to be a sensible view, namely that the spread of yellow fever could not be explained by the assumption of a diffusible miasma in the atmosphere, but required the presence of an intermediate host, appears to have been first advanced by Dr. Josiah Clark Nott of Mobile, Alabama, in March 1818 Nott suggested the mosquito as a possible agent for the dissemination of both yellow fever and malaria Dr Louis Daniel Beauperthuy in Venezuela made a similar suggestion in 1854 The first really serious

proponent of the mosquito transmission of yellow fever was Dr. Carlos J. Finlay of Havana, Cuba. Dr. Walter Reed himself stated, "To Dr. Carlos J. Finlay, of Habana, must be given, however, full credit for the theory of the propagation of yellow fever by means of the mosquito, which he proposed in a paper read before the Royal Academy in that city at its session on the 14th day of August 1881." (Reed, 1901.)



FIG. 1. "The First Mountain to be Removed," by W. A. Rogers, 1905. (*Courtesy of Bettman Archive*.)

A number of workers at various times claimed to have discovered the cause of yellow fever. The only theory that received serious attention, however, was that of Dr. Giuseppe Sanarelli, an Italian bacteriologist working in the Island of Flores off Montevideo. He announced in June 1897 that he had discovered the cause of yellow fever to be a bacillus present in about 50 per cent of the patients examined by him (Sanarelli, 1897). Because of Dr. Sanarelli's high standing in the field of bacteriology, his announcement was taken seriously; in fact, his *Bacillus icteroides* was confirmed as the

causative agent of yellow fever by some other investigators notably some members of the United States Public Health Service (Wasdin and Geddings 1899)

The first research of Dr Walter Reed Surgeon United States Army (Fig 2) on the cause of yellow fever was a study of *B icteroides* He concluded that the bacillus was a secondary invader belonging to the colon group and was not the cause of yellow fever (Reed and Carroll 1899) In June 1900 Dr Reed was sent to Cuba as president of a commission appointed to study the infectious diseases of that country but more especially yellow fever On June 25 1900 Dr Reed and Acting Assistant Surgeon James Carroll arrived at Columbia Barracks Quemados Cuba where the two other members of the Yellow Fever Commission Acting Assistant Surgeons Jesse W Lazear and A Agramonte were stationed (Fig 3)



Fig 2 Major Walter Reed President of the Yellow Fever Commission of the United States Army 1900 (*Photographed from portrait courtesy of Brown Brothers*)

The town of Quemado at that time was in the throes of an epidemic of yellow fever and the four men immediately began observations of the clinical bacteriologic and pathologic aspects of the disease The assertion of Sanarelli that *B icteroides* was the etiologic agent of yellow fever had excited such wide notice that it seemed to Reed and his associates that it was of the first importance to give undivided attention to the isolation of this microorganism from the blood of yellow fever patients and from the blood and organs of yellow fever cadavers From their study of 18 clinical cases and 11 autopsies they concluded *Bacillus icteroides* (Sanarelli 1897) stands in no causative relation to yellow fever but when present should be considered as a secondary invader in this disease (Reed Carroll et al 1900)

Having failed to isolate *B icteroides* from the blood of either living yellow fever patients or those who had succumbed to the disease the

group considered two courses of procedure they could undertake further bacteriologic studies or they could give attention to Finlay's theory of the propagation of yellow fever by means of the mosquito (Finlay, 1881) In light of the known facts about the epidemiology of yellow fever, notably the observations made by Surgeon Henry Rose Carter in 1898 at Orwood



Fig 3 The United States Army Yellow Fever Commission formed in 1900 and headed by Major Walter Reed (A) Other members of the commission were Dr James Carroll (B) Dr Jesse W Lazear (C) and Dr Aristides Agramonte (D) (*Photographs adapted from bookplate courtesy of Brown Brothers*)

and Taylor, Mississippi, that the period from the first (infecting) case to the first group of cases infected at these houses is generally from two to three weeks,' the commission decided to take up the mosquito theory Their decision was also influenced by the work of Sir Ronald Ross and of Italian observers on the propagation of malaria by the mosquito

Dr Finlay (Fig 4) accorded the commission a most friendly interview and placed his publications at their disposal He also gave them some ova of the species of mosquito with which he had worked These ova promptly hatched when placed in water and from them was developed the mosquito colony with which the Reed commission carried out its work

In September 1900 the Reed commission recorded three cases of yellow fever transmitted by mosquitoes that had fed previously on patients clinically ill with yellow fever (Reed Carroll et al 1900) Subsequent work of the commission proved conclusively that (*a*) the mosquito was the vector of yellow fever (*b*) there was an interval of about twelve days between the time the mosquito took an infectious blood meal and the time it could convey the infection to another human being (*c*) yellow fever could be produced experimentally by the subcutaneous injection of blood taken from the general circulation of a yellow fever patient during the 1st and 2d days of his illness and (*d*) yellow fever was not conveyed by fomites In consequence of these findings Reed and his coworkers suggested that the spread of yellow fever could be most effectively controlled by antimosquito measures and the protection of the sick from the bites of mosquitoes (Reed Carroll and Agramonte 1901*a* and *b*) At this time Dr Reed stated While the mode of propagation of yellow fever has now been definitely determined the specific cause of the disease remains to be discovered



FIG 4 Dr Carlos Finlay who facilitated the work of the United States Army Yellow Fever Commission in Cuba (*Photograph adapted from bookplate, courtesy of Brown Brothers*)

Although the commission had demonstrated that the specific agent of yellow fever is present in the blood of patients all efforts at isolating the causative organism resulted in complete failure At this point Dr William H Welch of the Johns Hopkins University called Dr Reed s attention to the important observations of Loeffler and Frosch (1898) on the transmission of foot and mouth disease in cattle by means of serum that had been passed several times through a porcelain filter capable of preventing the passage

of the smallest known bacteria These investigators gave two possible explanations for their remarkable results either the filtered lymph contained in solution an extraordinarily active toxin or else the specific agent of the disease was so minute that it could pass through the finest filter They held to the latter explanation

On October 15 1901 Reed and Carroll injected subcutaneously 3 cc of diluted and filtered serum from an experimentally infected yellow fever patient into three nonimmune persons Two of these developed clinical yellow fever (Reed and Carroll 1902) Walter Reed stated these experiments appear to indicate that yellow fever is caused by a micro organism so minute in size that it might be designated as ultramicroscopic Thus for the first time a filtrable virus was proved to be the cause of a specific human disease



Fig 5 General William C Gorgas Surgeon General of the United States Army 1914–1918 Director of the Yellow Fever Commission of the International Health Board 1918 1920

For one hundred and fifty years prior to 1902 yellow fever had been constantly present in the city of Havana The conclusions of Dr Reed and his associates pointed so clearly to mosquito eradication as the practical method of exterminating yellow fever that the principle was at once accepted by the sanitary authorities in Cuba and put to test in Havana In February 1901 the chief sanitary officer in Havana then Major William C Gorgas (Fig 5) instituted measures to wipe out yellow fever which were based entirely on the conclusions of the Yellow Fever Commission

The results were as dramatic as the scientific findings of the commission By September 1901 the disease had been completely eradicated and it has not reappeared The antimosquito measures in Havana in addition to eliminating yellow fever greatly reduced the incidence of malaria From Havana Gorgas went to Panama to launch the second great demonstration in the control of yellow fever and malaria The Havana and Panama campaigns now constitute an epic chapter in the history of sanitation and preventive medicine

Thus shortly after the turn of the century the cause of the dreaded yellow fever had been established its mode of transmission had been de fined and methods for its control had been successfully demonstrated—all in the incredible space of approximately 3 years Optimism seemed justified and Major Gorgas in his report of July 12 1902 to Brigadier General Leonard Wood in Washington stated I look forward in the future to a time when yellow fever will have entirely disappeared as a disease to which mankind is subject At the time however yellow fever was present in Mexico Central America South America and Africa and New Orleans was yet to experience its last epidemic As we know now our knowledge of yellow fever was far from complete

THE ROCKEFELLER FOUNDATION YELLOW FEVER COMMISSIONS

This was the situation in 1913 when The Rockefeller Foundation was organized for the well being of mankind throughout the world The International Health Commission of the Foundation was created the same year with Mr Wickliffe Rose (Fig 6) as its director In the winter of 1913–1914 Mr Rose went abroad to discuss with health officials a possible program for the newly created commission He discovered that throughout the East there was great anxiety on account of the possibility of the intro duction of yellow fever into that region as a result of the opening of the Panama Canal The canal would bring about radical changes in trade rela tions Countries and ports between which there had been little or no exchange were to be brought into close contact Pest holes of infection once relatively harmless because of their isolation would be on or near the world s highways of commerce and travel As early as 1903 Manson called attention to the grave health risks to which the opening of the canal exposed the East Dr S P James of the Indian Medical Service made a thorough investigation of the situation on behalf of the Indian government and reported that the menace was sufficiently great to call for a permanent quarantine force in Panama Hong Kong or Singapore to be maintained at the expense of the English colonies in the East He also recommended to the Indian government a systematic attack on the mosquito Sanitarians recognized that if yellow fever were once introduced into the Orient with its dense population of nonimmunes incalculable damage would accrue

In July 1914 Mr Rose told Dr Gorgas by then Surgeon General of the

United States Army that the Panama Canal was giving people in the Far East considerable anxiety on the subject of yellow fever In the course of this conversation General Gorgas reiterated his belief that the eradication of yellow fever from the earth was feasible that it could be accomplished within a reasonable time and at a reasonable cost At this same meeting Mr Rose suggested and General Gorgas agreed that they should go ahead and draw up plans for wiping out yellow fever through attacks on the insect vector At a later conference in which Dr Henry Rose Carter and Dr Joseph H White also participated the eradication of yellow fever was unanimously approved as a practicable and worthy project for The Rockefeller Foundation to undertake



Fig 6 Mr Wickliffe Rose Director of the International Health Commission 1913–1916 Director of the International Health Board 1916–1923

A meeting of the International Health Commission was held at the offices of The Rockefeller Foundation in New York on May 26 1915 The Secretary of the Foundation reported that he had received from Major General William Crawford Gorgas Surgeon General of the United States Army a letter expressing his willingness to enter the service of The Rockefeller Foundation for yellow fever work The following resolution was then adopted

> Whereas yellow fever has been endemic in tropical and subtropical America for centuries constituting a serious menace in the infected areas and a perennial source from which epidemics have spread to more remote regions both in America and Europe involving great loss of life and interrupting industry and trade over vast areas and
>
> Whereas it has been shown by the work done in Havana and Panama under the direction of General William C Gorgas and in Rio de Janeiro under the direction of Oswaldo Cruz that the infection can be eradicated even in communities where it is endemic and

Whereas the opening of the Panama Canal and the changing of trade relations resulting therefrom have given rise to widespread apprehension that yellow fever may be introduced into the Orient and that once endemic in these densely populated regions it would become a permanent menace to the rest of the world therefore

Be it resolved that the International Health Commission is prepared to give aid in the eradication of this disease in those areas where the infection is endemic and where conditions would seem to invite cooperation for its control

The meeting also adopted a resolution appointing General Gorgas director of the commission s work for the eradication of yellow fever

It should be borne in mind that at that time the epidemiologic concept of yellow fever was a simple one namely that the disease could be acquired only through the bite of an *A aegypti* mosquito that had become infected by feeding on a human being sick with yellow fever that there were certain endemic centers of the disease that served as seedbeds that these foci of infection were few in number, and that if they were destroyed yellow fever would disappear forever

In 1916 the newly established Rockefeller Foundation Yellow Fever Commission consisting of General Gorgas Chairman Dr Henry Rose Carter Dr Juan Guiteras Major T C Lyster Major E R Whitmore and Mr W D Wrightson (Fig 7) visited suspected endemic centers in Ecuador Peru Colombia and Venezuela Later in the same year the commission also visited the principal ports of Brazil from Rio de Janeiro to Para From their observations on these trips the commission concluded that the only endemic center in South America was in Guayaquil Ecuador They recommended that the infection be eliminated from Guayaquil that the east coast of Brazil and the southern littoral of the Caribbean be kept under observation and that investigations be extended to Mexico and West Africa both of which regions were under suspicion

The actual campaign for the eradication of yellow fever from Guayaquil was delayed by the entry of the United States into the First World War which required General Gorgas to devote himself exclusively to his duties as Surgeon General until he retired in 1918 Preliminary work however did get under way Before plunging into a campaign to clear Guayaquil of yellow fever in accordance with the recommendation of the Yellow Fever Commission it seemed wise to learn more about the disease there A special commission arrived in Guayaquil in July 1918 and carried on

intensive investigations for about two months The commission whose members were Dr Arthur I Kendall Dr Hideyo Noguchi Dr Mario G Lebredo Dr Charles A Elliott and Mr Herman E Redenbaugh included experts in clinical medicine epidemiology bacteriology and chemistry



Fig 7 Members of The Rockefeller Foundation Yellow Fever Commission 1916–1918 Front row left to right Major Lyster Dr Guiteras General Gorgas Dr Carter Back row Mr Wrightson and Major Whitmore

The commission left Guayaquil early in September 1918 except Dr Noguchi who stayed on until the end of October

The report of the commission was completely overshadowed by Dr Noguchi s announcement of his discovery of a leptospira that in guinea pigs produced lesions suggestive of yellow fever (Noguchi 1919*a*) Later Noguchi and other investigators obtained the same organism in yellow fever epidemics in Mexico Peru and Brazil After much experimental work Noguchi decided (erroneously as was proved later) that this lepto

spira was the cause of yellow fever and named it *Leptospira icter*
(Noguchi 1925) The findings of Walter Reed and his associates in Ha
(United States 61st Congress Yellow Fever 1911) had been fully
firmed and amplified by Guiteras of Cuba (1901) Ribas of Sao Paulo (19
Barreto de Barros and Rodrigues of Brazil (1903) Parker Beyer
Pothier (1903) Rosenau Parker et al (1904) Rosenau and Goldber
(1906) Marchoux Salimbeni and Simond (1903) Marchoux and Simo
(1906*a* *b* and *c*) and Otto and Neumann (1905) The contradictory ev
dence of so distinguished a bacteriologist as Noguchi together with the con
firmation of his findings by other workers again confused the picture of th
etiology of yellow fever Dr Noguchi s error is easily understandable
Clinically yellow fever and spirochetal jaundice are practically indistin
guishable and in addition the two diseases sometimes are present at the
same time as was the case at Guayaquil and the other places that Dr
Noguchi investigated

The full scale campaign for the eradication of yellow fever from Guaya
quil was started in 1918 Dr M E Connor a member of the field staff
of the International Health Board (which had superseded the International
Health Commission in 1916) arrived there early in November According
to the plan agreed upon Dr Connor was made director of yellow fever
eradication under the Director General of Health of Ecuador and on No
vember 27 active operations began By the end of December 125 men had
been engaged and 25 mosquito squads were at work In November there
were in Guayaquil 77 recorded cases of yellow fever and in December
there were 86 cases there were 78 in January 1919 37 in February 13 in
March 2 in April 1 in May and 1 in June Thereafter and to date no
cases have been recorded For the first time in approximately one hundred
years the city was free of yellow fever This project marked the first effort
of The Rockefeller Foundation at yellow fever control through anti
mosquito measures The lifting of the long standing quarantine against
Guayaquil in 1920 attested to its success

In June 1918 the disease became epidemic in Guatemala and during
919 outbreaks were reported from Peru and Brazil in South America
om Honduras El Salvador and Nicaragua in Central America and from
Mexico The success of the Guayaquil work had attracted wide attention
nd the governments of El Salvador Nicaragua Honduras Guatemala
d Mexico invited the International Health Board to join with them in
orts to control the disease The invitation was accepted and the out

breaks were quickly suppressed through use of the techniques established at Guayaquil Yellow fever became epidemic in Peru in 1920 and in February 1921 the International Health Board in cooperation with the Peruvian government began control operations in that country Early in 1923 several hundred cases of yellow fever were reported at Bucaramanga the capital of the Department of Santander in Colombia On invitation from the Colombian government control measures were instituted there

Although Oswaldo Cruz (Fig 8) and his associates were successful in controlling epidemics of yellow fever in Rio de Janeiro and in Santos the disease was prevalent elsewhere in Brazil The country had a yellow fever area about 1 500 miles in length extending from Santos up the coast to Belem and from the mouth of the Amazon to the Peruvian frontier Much of this region had from time to time experienced severe epidemics In May 1923 the federal government of Brazil invited the International Health Board s collaboration in connection with the government s yellow fever control program This invitation was also accepted and control measures began there the same year By the end of 1924 the disease had been eradicated from Mexico from Central America and from Ecuador and the other trouble areas in South America were being attacked



Fig 8 Dr Oswaldo Cruz pioneer in the control of yellow fever in Brazil (*Photographed from portrait*)

Although the Yellow Fever Commission that went to South America recommended in 1916 that the west coast of Africa be investigated the First World War delayed the implementation of this recommendation until 1920 In June of that year the Yellow Fever Commission to the West Coast of Africa headed by General Gorgas sailed from New York The other members of this group were Dr R F Noble Assistant Surgeon General United States Army Dr Juan Guiteras Director of Public Health of Cuba Dr Adrian Stokes assistant to the Professor of Pathology Trinity

College Dublin Dr A E Horn of the West African Medical Service and Dr W F Tytler of the staff of the Medical Research Council of Great Britain The group with the exception of General Gorgas arrived in Lagos Nigeria on July 17 1920 En route General Gorgas was stricken with a fatal illness and died in London on July 4 While General Gorgas was in the hospital in London King George made him Knight Commander of the Order of St Michael and St George in recognition of his services After General Gorgas s death the commission was headed by General Noble



Fig 9 West Africa Yellow Fever Commission quarters circa 1925

The objectives of the commission to West Africa were two to determine whether the reported yellow fever in that region actually was yellow fever and if so to ascertain whether control measures were feasible From Lagos the commission visited Dahomey the Gold Coast Senegal Sierra Leone and Matadi in the Belgian Congo No authentic cases of yellow fever were seen but the investigation indicated strongly that the disease had been present within recent years The region of suspected infection was vast travel was difficult and living conditions were extremely primitive Even under these conditions however the control of yellow fever was not regarded as entirely impracticable The commission recommended that its report be accepted merely as a progress report and that another more fully equipped commission be appointed to carry out a more extensive and prolonged investigation of the situation in West Africa including a laboratory study of the suspected fevers of the region

Accordingly in 1925 still another commission under the leadership of Dr Henry Beeuwkes was sent to West Africa (Fig 9) This commission known as the West Africa Yellow Fever Commission had specific objec

tives in mind namely (*a*) to study the characteristics and epidemiology of the disease in West Africa and its relationship to the yellow fever of the Western Hemisphere (*b*) to isolate the organism that caused the disease (*c*) to discover the method of transmission and (*d*) to identify those areas in which the disease was continually present

During the first 2 years of the commission s operations many cases of yellow fever were investigated and the bacteriology pathology and symptomatology as well as the possible vectors of the disease studied In an exhaustive study of 67 cases of yellow fever the commission failed to isolate Noguchi s *L icteroides* In the mean time doubt as to the validity of Noguchi s finding had also arisen in a number of other quarters—Borges Vieira in Brazil Lebredo working in Yucatán Guiteras in Cuba (1921*a*) Agramonte in Cuba (1924) and Theiler and Sellards in the United States of America (1926)



Fig 10 Asibi West African yellow fever survivor whose blood sample provided virus material for extensive yellow fever research

Laboratory work on the disease was very much handicapped by the lack of an experimental animal This was so important an obstacle at this juncture that the group in West Africa concentrated on trying to overcome it At the end of June 1927 Dr A F Mahaffy a member of the commission stationed at Accra investigated cases reported among the native population of Kpeve He obtained blood specimens from two patients suspected of having mild infections one of whom was a 28 year old African man named Asibi (Fig 10) Drs Mahaffy and Bauer of the commission s laboratory staff inoculated this blood into one rhesus monkey (Fig 15) one marmoset and two guinea pigs The rhesus monkey developed fever on July 4 the 4th day after inoculation and was found moribund on the following morning This first experimental transmission of the virus of yellow fever to an animal other than man opened up entirely new possibilities for laboratory and field research Propagation

of the now famous Asibi strain of yellow fever virus also began w
experiment (Stokes Bauer and Hudson 1928a)

Also in 1927 these same workers confirmed definitively that the ca
agent of yellow fever was a filtrable virus They demonstrated in ad
that the infection was easily transmitted from monkey to monkey as
as from man to monkey by the injection of citrated blood taken ear
the disease that it was easily transmitted from monkey to monkey
A aegypti mosquitoes that once infected mosquitoes remained infec
for the entire period of their lives which in some instances exceede
months and that the bite of a single infected mosquito was sufficient
produce a fatal infection in a monkey (Stokes Bauer and Hudson 192
and *b*) The year 1927 was indeed a fruitful one in the annals of yello
fever research

At this time it was generally believed that *A aegypti* was the only vecto
of yellow fever in the Western Hemisphere for the disease had been elimi
nated from most of the hemisphere by the control of this one mosquito
In numerous experiments conducted by early workers yellow fever was
transmitted from man to man by these mosquitoes while similar experi
ments with other species proved negative Marchoux and Simond (1906a
and *b*) attempted to transmit the disease by five different mosquitoes *Aedes
scapularis* *Aedes taeniorhynchus* *Culex quinquefasciatus* *Psorophora cili
ata* and *Psorophora posticata* with uniformly negative results

In West Africa there are numerous species of the genus *Aedes* which
resemble aegypti very closely in their bionomics these species are not
found in America In 1928 Dr J H Bauer of the West African group
reported the transmission of yellow fever by two of these species *Aedes
luteocephalus* and *Aedes apicoannulatus* and also by *Eretmapodites chryso
gaster* (Bauer 1928) This was the first indication that the epidemiology
of yellow fever might not be so simple as previously believed and that
more was involved than the mere transmission of the disease from man to
A aegypti to man The following year Cornelius B Philip entomologist
with the West Africa commission reported the transmission of yellow fever
in monkeys by three additional species of the genus *Aedes* *Aedes vittatus*
Aedes africanus and *Aedes simpsoni* (Philip 1929a) The same year Davis
and Shannon of the staff of the Foundation s International Health Division
which superseded the International Health Board in 1927) working in
Brazil reported the transmission of yellow fever by *Aedes (Ochlerotatus)
apularis* (Davis and Shannon 1929b) Also in 1929 workers in Java

reported the transmission of yellow fever with *Aedes (Stegomyia) albopictus* (Dinger Schuffner et al 1929) Philip in West Africa extended his observations and found that a mosquito of a genus other than *Aedes* namely *Taeniorhynchus (Mansonioides) africanus* Theobald could also transmit the disease (Philip 1930*a* *b* and *c*)

THE YELLOW FEVER LABORATORY IN NEW YORK

The findings of the West Africa commission proved conclusively that yellow fever in that area was due to a filtrable virus and all the evidence pointed to the fact that yellow fever in Africa and yellow fever in the Western Hemisphere were one and the same disease Some workers however remained unconvinced and persisted in the opinion that there were two clinically similar but nonetheless separate diseases one caused by a filtrable virus and the other by *L ic teroides* When Brigadier General Frederick F Russell (Fig 11) succeeded Mr Wickliffe Rose as Director of the International Health Board in 1923 he took note of this controversy and decided that the best way to settle it would be to bring together in a single laboratory preferably away from the infected countries the yellow fever viruses of Africa and South America and make cross immunity tests in monkeys Through the courtesy of Dr Simon Flexner space in The Rockefeller Institute for Medical Research in New York was made available to the International Health Division for the establishment of a yellow fever laboratory Field laboratories were already operating in West Africa and in Brazil and the New York laboratory was opened on June 24 1928 under the direction of Dr Wilbur A Sawyer (Fig 12)



Fig 11 Dr Frederick F Russell Director of the International Health Board 1923–1927 Director of the International Health Division 1927–1935

At the outset Dr Sawyer was faced with some difficulties in organizing the work He wrote The recent tragic deaths of Stokes Noguchi and Young were fresh in mind and had shown how great a risk was run in yellow fever research even by the most skilled of laboratory workers It was accordingly decided for safety that only medical men should be allowed to participate in the work during the early stages With full realization of the danger Dr Wray Lloyd and Dr S F Kitchen both of the University of Western Ontario agreed to take part Together we carried out the experiments fed the monkeys took their temperatures cleaned and sterilized their cages performed the necropsies when they died and incinerated the bodies and rubbish All through the hot summer months we labored thus in mosquito proof rooms with screened vestibules In October Dr Martin Frobisher Jr formerly instructor in the Johns Hopkins Medical School joined the staff and it was decided that it would be permissible to employ mature assistants to help with the animals and do technical work if they were fully informed of the danger they would run In most cases the blacker we painted the picture the more eager were the applicants to take the positions With this change the work of the laboratory became less arduous and the output in results became greater (Sawyer 1931*c*)



Fig 12 Dr Wilbur A Sawyer Director of the International Health Division 1935 1944

Strains of virus were obtained from West Africa (Asibi strain) from Dr A W Sellards and from Dr Max Theiler of Harvard University (the French strain) and from Dr H de B Aragao of the Oswaldo Cruz Institute (the F W strain) Cross immunity tests with these viruses were decisive as were supplementary tests of various sera for their protective powers against yellow fever virus African and American yellow fever were one and the same Additional evidence of the identity of the African and the American yellow fever was obtained by testing sera from yellow fever

immune persons in each region for protective power against the virus of the other regions (Sawyer Kitchen et al 1930)

The rhesus monkeys used in all these experiments were not only hard to handle because of their large size but were also expensive to purchase and maintain The International Health Division urgently needed a more suitable experimental animal for protection tests in extensive field investigations and in immunity surveys At this point Dr Theiler of the Department of Tropical Medicine of the Harvard Medical School working with the French strain of virus made the important discovery that white mice were susceptible to yellow fever if inoculated intracerebrally and that a fixed virus for mice with a shortened incubation period and heightened virulence could be produced by repeated passage through these animals (Theiler 1930*a*) In a later publication Theiler described the use of mice in testing sera for protective substances against yellow fever virus (Theiler 1931) This mouse protection test of Theiler s as modified by Sawyer and Lloyd became one of the principal tools in yellow fever research and epidemiologic investigations (Sawyer and Lloyd 1931)



Fig 13 Dr George K Strode Director of the International Health Division 1944–1951

In spite of improved laboratory techniques and the most stringent precautions laboratory and field investigations on yellow fever were not without risk In 1931 Berry and Kitchen reported 32 cases of laboratory infection with yellow fever virus and five deaths (Berry and Kitchen 1931) Cases had occurred in the International Health Division laboratories in West Africa and Bahia Brazil and in the New York laboratory with deaths in the African and Brazilian laboratories This situation deeply disturbed both Dr Russell the Director of the International Health Division and Dr Sawyer the head of the New York laboratory Some form of protective immunization was obviously in order Dr Sawyer and his associates as well as other investigators observed that monkeys inoculated

with highly virulent strains of yellow fever virus 1 to 6 hours following an injection of yellow fever immune serum possessed a solid active immunity after the passive immunity had disappeared On the basis of this observation, Dr Sawyer, using a less virulent strain established in mice by Theiler, devised a vaccine consisting of a 10 per cent suspension of infected mouse brain tissue in fresh, sterile human immune serum This material, when used with supplementary immune serum, gave solid immunity in monkeys without the development of symptoms After thorough testing in monkeys, 10 persons were vaccinated between May 13 and June 29, 1931 (Sawyer, Kitchen, and Lloyd 1931) This was the first vaccine against yellow fever, and after its introduction no further cases of the disease occurred in the International Health Division laboratories

THE DISCOVERY OF JUNGLE YELLOW FEVER

Students of yellow fever had considered the disease to be essentially urban in character with *A aegypti* necessary for its transmission under natural conditions This concept was strengthened by the dramatic disappearance of yellow fever from all well known centers of infection on the American continents following the application of antimosquito measures directed against *A aegypti* Although Bauer (1928) and Philip (1929*a*, 1930*a*, *b*, and *c*) in Africa and Davis and Shannon (1929*b*, 1931*a* and *b*) in South America had shown that other species of mosquitoes could transmit yellow fever virus from animal to animal in the laboratory the concept of an epidemic even of small proportions in the absence of *A aegypti* had not gained acceptance

In 1907 an epidemic in Muzo, Colombia, was investigated by Dr Robe[...] Franco and his associates They concluded that the epidemic was due [...] yellow fever and an associated fever of spirochetal origin and that th[...] diseases existed endemically in the region, becoming epidemic at t[...] with the arrival of susceptible individuals from the cold uplands In des[...] ing this outbreak, they stated, 'The yellow fever [observed here] has ce[...] peculiarities from an etiological point of view, (*a*) It is contracted [...] forest and not in the neighborhood of the houses, (*b*) It is transmit[...] *Stegomyia calopus* and probably also by other culicines, (*c*) Inoc[...] takes place during the daylight hours, which are spent by the wor[...] places where the transmitting mosquitoes predominate ' (Franco, M[...]

Santamaría and Toro Villa 1911) Dr Fred L Soper in a lecture before the Faculty of Medicine of Bogotá in April 1935 stated "Dr Franco has really given us a quarter of a century ago an excellent description of jungle yellow fever acquired in the forest and believed by him to have been transmitted by a non domestic mosquito whose habits are described" (Soper 1935b)

From January to April 1932 there was a wholly rural epidemic of yellow fever in the Valle do Chanaan Espirito Santo Brazil in which *A aegypti* could definitely be ruled out as the vector The epidemic was described in detail by Soper Penna et al (1933) This epidemic definitely modified the epidemiologic concept of yellow fever on two counts (*a*) it was a strictly rural outbreak and (*b*) *A aegypti* played no part in its propagation It also presented new problems to be solved if the disease was to be eradicated or even brought under control The complexity of the problem was further accentuated by the repeated detection of yellow fever of jungle origin in Brazil by Soper (1936b) Soper in his definition of jungle yellow fever stated that it is "yellow fever occurring in rural jungle and fluvial zones in the absence of *A aegypti*" He continued

The epidemiologic picture of jungle yellow fever is quite different from that of urban and rural yellow fever transmitted by *A aegypti* Yellow fever transmitted by *A aegypti* is characteristically a "house disease" and occurs indiscriminately among nonimmunes living in or visiting infected houses It apparently depends upon the simple cycle man mosquito man for its maintenance and is spread from one point to another either by the movement of the human host during the period of incubation or by the accidental transportation of the infected mosquito from place to place

Infection with jungle yellow fever is believed to take place generally in or close to uncut forest or jungle In certain areas where the fields are next to the jungle and at some distance from the farm houses cases are limited largely to those who work in the fields or visit them In other places where the entire population lives in more direct contact with the jungle infections appear to have a more general distribution throughout the entire population

Up to the present time yellow fever without *Aedes aegypti* has been observed only in areas where the clearing of the forest has been incomplete although in some areas of jungle yellow fever the percentage of land covered by jungle or forest growth is very small in comparison with the total area

Jungle yellow fever is different from the aegypti transmitted form in that it has been found under conditions suggesting that infections take place away

from the houses and that man may not be an essential factor in the continu ance of endemicity nor in the spread of the virus from one place to another Isolated cases occur in such a way as to indicate that jungle yellow fever depends upon other factors than the simple man mosquito man cycle of the aegypti transmitted disease and to suggest that jungle yellow fever as seen in man may be but an accident in the course of an epizootic in the lower animals or may even be due to the persistence of the virus in invertebrate vectors for long periods of time

The perfection of the mouse protection test and the fact that an attack of yellow fever is followed by an enduring immunity with protective anti bodies in the blood made it possible to undertake a worldwide survey to determine the distribution of yellow fever (Sawyer 1931*b*) The first yellow fever immunity survey was made in Nigeria by Beeuwkes Bauer and Mahaffy (1930) At this time since only monkeys were available for immunity tests and since it was necessary to use two rhesus monkeys for each serum tested the number of bloods that could be examined was quite limited This handicap was removed when the mouse protection test became avail able and in 1931 a systematic survey of yellow fever immunity around the globe was begun The results published in a number of papers showed that the virus of yellow fever had remained inactive in most parts of the world and at the outbreak of the Second World War was active only in restricted areas of Africa and South America

The mouse protection test could not do more than provide workers with *post facto* evidence of yellow fever prevalence For the purpose of control operations it was desirable to have more up to date information Most jungle *yellow fever occurs in isolated areas with poor communications* where medical services are either extremely sparse or nonexistent In an effort to obtain information on the actual occurrence of the disease Dr R R Rickard while working in Brazil devised an instrument for the removal of fragments of liver for histopathologic examination without autopsy (Rickard 1931) This instrument is called the viscerotome It can be used by nonmedical personnel and was employed effectively on a nation wide scale in Brazil and also in other countries (Soper Rickard and Craw ford 1934 Rickard 1937)

SPECIES SANITATION

Antimosquito measures for the control of disease were first used by Gorgas in Havana in 1901. While the specific objective of Gorgas was the control of yellow fever, his efforts were aimed at mosquitoes in general. Yellow fever was eliminated and malaria was reduced. Measures for the general control of mosquitoes are, as a rule, expensive and are unnecessary in the control of either yellow fever or malaria. Species sanitation was first used by Malcolm Watson in 1911 in malaria control in the Federated Malay States. In aegypti sanitation it is not necessary to eradicate the species in order to prevent the transmission of yellow fever. As aegypti density is lowered by antilarval measures the number of imagoes present eventually becomes too small for the virus to maintain itself, and yellow fever gradually dies out in the controlled area (Carter, 1931). Yellow fever was successfully controlled in a great many cities by this procedure.

Experience also showed that when active control measures were relaxed, aegypti production increased rapidly. As long as the man–mosquito–man concept of yellow fever epidemiology prevailed, urban centers usually felt secure enough to relax their control measures, once the disease had been eliminated. That this procedure was not entirely safe became evident even before the recognition of jungle yellow fever, for in 1928 yellow fever reappeared in Rio de Janeiro after an absence of 20 years. As soon as jungle yellow fever was discovered in Brazil, it became obvious that no urban center in that country could relax its anti aegypti programs. It seemed that these cities might be faced with the problem of aegypti control in perpetuity. To cope with this dilemma Soper and Wilson (1942) decided that the answer was species eradication. They stated, "Species eradication should be defined as the *complete extermination of the species under consideration* in all phases of its development: ovum, larva, pupa and imago. When species eradication has been accomplished, the crucial test of discontinuation of all control measures, in the presence of suitable breeding places, is not followed, in the absence of reimportation, by reappearance of the species." Soper, Wilson et al. (1943) developed and applied species eradication techniques in Brazil with brilliant success.

THE RIDDLE OF TRANSMISSION

Jungle yellow fever was a new and vexing problem How was it transmitted? What were the vectors? How was the virus maintained in nature? These were only three of many unknowns about this new phase of yellow fever The laboratories and epidemiologists in Rio de Janeiro, Bogotá, Colombia, Entebbe, Uganda, and Lagos, Nigeria all tackled the problem

Epidemiologists in Brazil noted that jungle yellow fever occurred more often in men than in women, especially in robust individuals whose work had some relation to the forest, such as woodcutters and road builders The infection seemed to be acquired during the day rather than at night Secondary cases were not as common as in aegypti transmitted yellow fever. Investigations carried forward in Brazil indicated that the vector was able to fly but there was no definite knowledge of this It was felt likely that the cycle in nature involved arthropods of some kind and perhaps various mammals To account for the endemicity some form of reservoir was postulated Evidence accumulated in Brazil, Colombia, and Africa indicated that the mechanism of yellow fever maintenance in the forest was probably a mammal—mosquito cycle in which man was only incidentally involved when he projected himself into the picture Studies on the epidemiology of yellow fever in the forest were therefore largely concentrated on attempts to isolate the virus from mosquitoes and to determine the extent of immunity among mammals

One particular aedine mosquito, *Haemagogus spegazzinii (capricornii)*, was an early suspect in both Brazil and Colombia This species, however, seemed too scarce, and attempts to transmit the virus with this mosquito in the laboratory met with but little success The mosquitoes were difficult to keep alive under laboratory conditions, and even when they could be kept alive for what seemed an adequate period transmission did not occur or was irregular In 1938 however, workers in Brazil reported the transmission of yellow fever with a naturally infected, wild caught *H. spegazzinii (capricornii)* (Shannon, Whitman, and Franca, 1938)

In 1940 workers in Colombia organized a mobile field laboratory to study jungle yellow fever on the spot wherever it might be reported (Bugher, Boshell Manrique, et al, 1944) In November 1940 two cases of yellow fever were reported from the headwaters of the Rio Ocoa and a

camp was established at La Cuchilla On November 18 1940 there was made an observation with respect to the behavior of *H spegazzinii* (*capricornii*) destined to change the entire orientation of the studies In descending the mountain the party encountered some woodcutters who were felling a tree (Fig 14) Since it had been noted that at this time in the forenoon there were very few haemagogus the group paused to see what would happen after the tree fell As the tree crashed to the ground tearing branches from its neighbors great numbers of haemagogus were observed viciously attacking the men It was then realized that these mosquitoes must have been present in the upper foliage and had been caused to fly by the disturbance of their environment It was evident that this finding would go far to explain the irregular catches of haemagogus at ground level In the course of time this original observation was amply confirmed It became routine to make catches of mosquitoes in the treetops as well as at ground level



Fig 14 Woodcutter at work a common method of contracting jungle yellow fever in eastern Colombia

Since haemagogus could now be collected in large numbers the yellow fever investigators were able to extend and intensify transmission experiments with this species in the laboratory But attempts to obtain transmission under controlled laboratory conditions continued to give anomalous results The group working on this problem in Colombia had been using the indigenous haemagogus mosquito the rhesus monkey an exotic mammal (Fig 15) and a strain of virus that had been subject to a lot of unnatural laboratory manipulations (Bates and Roca Garcia 1946*a*) Sporadic cases of jungle yellow fever continued to appear in the area and it occurred to the men that should they attempt in the laboratory to duplicate conditions of the locales where the disease was occurring naturally their efforts might be more successful A local strain of virus was recovered from a human

patient and inoculated directly into a local saimiri monkey *Saimiri sciureus caquetensis* The monkey which died 9 days later served as the source animal for a strain of virus that was kept going constantly in the laboratory by haemagogus transmission from monkey to monkey for a year With this new virus and the maintenance of haemagogus under laboratory conditions simulating their native tree top habitat there was no trouble at all in obtaining transmission in the laboratory



Fig 15 *Macacus rhesus* whose high susceptibility to yellow fever made it invaluable in experimental transmission studies in West Africa

In Brazil and Colombia adult monkeys showed a high degree of immunity to yellow fever as indicated by the mouse protection test Since both the monkeys and haemagogus were essentially arboreal in habit the facts fitted perfectly the theory of the mammal—mosquito cycle of forest propagation of the disease There was nevertheless an important missing link

An intensive epidemiologic study of the disease was undertaken in Brazil in the vicinity of Ilhéus Here the periodic occurrence of proved yellow fever among rural inhabitants over a protracted period in the absence of *A aegypti* justified the conclusion that jungle yellow fever existed in endemic form The investigations included extensive capture of wild animals for the purpose of determining the incidence of specific yellow fever immunity and also of demonstrating if possible the presence of virus (Taylor and Fonseca da Cunha 1946 and Laemmert de Castro Ferreira and Taylor 1946) A marmoset was captured on June 7 1944 On the following day when it was received at the field laboratory it was in a weak condition A blood sample was withdrawn from the heart on June 8 and 4 hours later the animal died On autopsy the macroscopic appearance was recorded as suspicious for yellow fever This diagnosis was confirmed by histologic examination Subinoculation from this marmoset resulted in the isolation of yellow fever virus (Laemmert and de Castro

Ferreira 1945) In August of the same year the virus was isolated from three additional marmosets in the same region

At the time of this epizootic and in the same locality the virus was also obtained from *H spegazzinii* The only other mosquito found infected in nature in Brazil and capable of transmitting the infection by bite *Aedes leucocelaenus* occurred in the area but in such small numbers as to rule it out as a likely vector in the Ilheus region

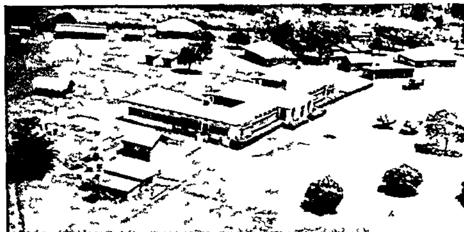


Fig 16 Yellow fever laboratory in Entebbe Uganda East Africa

The hypothesis of the mosquito—primate cycle of jungle yellow fever was thus corroborated by factual evidence in both Brazil and Colombia

Studies of rural yellow fever in East Africa were begun late in 1936 A central laboratory was organized in Entebbe Uganda in cooperation with the government of that protectorate and the Colonial Office in London An area in western Uganda Bwamba County was selected as the field area for intensive study When the studies in Uganda were inaugurated neither urban nor jungle yellow fever had been reported from that part of Africa although the yellow fever immunity survey reported by Sawyer and Whitman (1936) revealed a much wider distribution of immunity than could have been anticipated from the history of the disease A first problem was to determine if the disease extended that far east In South America the disease was known to be associated with the forest and especially with disturbance of the forest Since a road was being built through the virgin Semliki forest it was thought this would provide an excellent opportunity

Toro district east of Bwamba This quickly created an immune zone around the area of recent infection Bwamba was later included in the program of immunization

The origin of the inhabitants of Bwamba is obscure Some believe they sprang from an autochthonous race of forest pygmies others think they represent the most primitive of the Bantu tribes in Uganda Their mode of living is primitive there is no tribal unity habitations are scattered through the forest and communications are very poor It was therefore difficult to ascertain the state of affairs in regard to illnesses It seems reasonably sure however that yellow fever in epidemic form did not exist But in spite of the mass immunization campaign the virus continued active in both the humans and primates of the area This was evident from the fact that protection tests in children born in the area after the mass immunization campaign revealed a high incidence of immunity children 1 year old were 1 4 per cent immune, those 3 years old 61 3 per cent 4 years old 81 7 per cent and 5 years old 90 9 per cent Also 11 months after the vaccination campaign virus was again isolated from *A simpsoni* As it seemed almost certain that the virus in this case must have come from wild animals investigators turned attention to the main forest in the hope of finding the vector responsible for the transmission of yellow fever virus in forested areas uninhabited by man (Haddow 1915*b*) Monkeys classified by age and tested for immunity gave results as follows juvenile 4 per cent immune subadult 30 per cent adult 78 per cent old 100 per cent (Haddow Smithburn et al 1947)

What was the mode of transmission?

Observations by Haddow and others on the mosquitoes of Bwamba with particular reference to microclimate biting cycle and vertical distribution led to the conclusion that the mosquito most likely to be involved in the transmission of yellow fever among the monkeys of that area was *A africanus* This species was widely distributed in the central African forests In Uganda it survives the dry season in the adult state in forested and wooded areas and is an efficient vector of yellow fever in the laboratory Africanus shows a well marked peak of biting activity just after sunset in the forest canopy which is its preferred habitat This is a point of importance as monkeys normally repair before sunset to trees in which they pass the night and are asleep by the time *A africanus* reaches its highest activity (Haddow and Mahaffy 1949) Large scale catches of this species in the trees were made in the hope of isolating virus from them after allowing them to feed on

nonimmune rhesus monkeys and then triturating them and inoculati them into laboratory animals These efforts were unsuccessful

At this stage it was concluded that although yellow fever virus was prob ably constantly active it was very unlikely to be continuously active in any particular locality It therefore seemed uneconomical to continue large scale catches without definite evidence of the presence of virus in the area at the time concerned In August and September of 1945 16 nonimmune rhesus monkeys were stationed as sentinels in the forest canopy The animals were kept in cages especially designed to afford shelter and protection from predators but free access to blood sucking insects The cages were suspended by wires above platforms constructed in the trees from 50 to 60 feet above ground The animals were visited daily and their cages cleaned they were fed and their temperatures taken During 1946 the number of forest canopy sentinels was increased to 33 and in 1947 6 more were added By late 1947 none of these animals had become infected Then it was discovered that *A africanus* would not enter a cage to bite a monkey although it attacked both Indian monkeys and human beings avidly in the open (Haddow and Dick 1948) It was obvious therefore that the sentinels would have to be stationed in the forest canopy without cages This time consuming modifi cation was completed in February 1948 The effort was rewarded with suc cess In June 1948 the first sentinel developed fatal yellow fever and the virus was isolated Three additional fatal cases developed in the sentinels and the virus was recovered from each The *A africanus* caught in the area delineated by the sentinel monkey program produced yellow fever in nor mal rhesus monkeys both by biting and when ground up and inoculated into the monkey (Smithburn Haddow and Lumsden 1949)

In South America human jungle yellow fever occurs when man is bitten in the forest by the sylvan vector This vector is an arboreal insect wh ordinarily bites monkeys but may attack man when he fells the trees or otherwise comes in contact with the forest Jungle yellow fever in So America is an occupational disease with the same vector for the simian a human hosts

In central Africa sylvan yellow fever virus reaches the human host ightly different but roughly similar manner It is known that at least two ecies of monkeys both widely distributed and both susceptible to er commonly raid cultivated areas outside the forest and thus mselves to bites by *A simpsoni* a proved vector to human It lso known that this mosquito occasionally leaves its normal at in

plantation areas and proceeds into the canopy of the forest edge, where it may have an opportunity to bite infected monkeys *A africanus*, the sylvan vector, exhibits its optimum biting level at some distance above ground in the main forest canopy and its period of maximum biting activity at a time when human beings are not likely to be in the forest It seems likely that yellow fever in Africa as exemplified by the Bwamba country, is dicyclic, *that the forest vector does not ordinarily transmit the virus to man but* that men usually acquire the infection from *A simpsoni*, which in its turn acquires its infection from a monkey, either the insect or the simian host having temporarily left its usual environment It is also probable that in central Africa the reservoir vector of yellow fever virus is *A africanus*, since the climatic conditions permit year round survival of the adult of this species

DEVELOPMENT OF THE VACCINE

With the discovery of jungle yellow fever and the development of sub sequent knowledge of its epidemiology, the eradication of yellow fever seemed more remote than ever Both the mode of transmission and the reservoir host were unknown, the man–mosquito–man cycle definitely did *not fit the epidemiologic picture The virus lurking in the forest seemed* to be a constant threat to adjacent urban centers This situation urgently called for some practicable method of immunizing large populations The vaccine available (Sawyer, Kitchen, and Lloyd, 1932) gave a good solid pro tection, but since it required large quantities of sterile human immune serum for its administration, it was not practical for large scale use

In the Yellow Fever Laboratory in New York various strains of yellow fever virus were being cultivated in vitro One of these, the Asibi strain had been maintained for more than 3 years without intervening passage through an animal host (Lloyd, Theiler, and Ricci, 1936) The first step was successful establishment of the *Asibi strain in a culture medium con* taining embryonic mouse tissue and 10 per cent normal monkey serum in Tyrode s solution After cultivation through 18 subcultures in this medium *cultivation of a separate* branch of this virus was initiated in a medium con taining minced whole chick embryo After 58 subcultures in the latter me dium, the tissue component of the medium was modified by removing the brain and spinal cord from the chick embryo before mincing The virus was later maintained continuously in this medium for over 160 subcultures The resultant strain was designated as 17D

In 1937 Theiler and Smith reported that both the viscerotropic neurotropic virulence of this virus cultivated in chick embryo had b markedly reduced When injected subcutaneously into monkeys it p duced a mild generalized infection demonstrated by minimal quantit of virus found circulating in the blood When injected into monkeys intr cerebrally it rarely produced a fatal encephalitis but only a moderat febrile reaction followed by recovery and a solid immunity against a tes strain of highly virulent virus The same year Theiler and Smith reported on the use of this modified virus 17D for human immunization

In January 1937 Dr Smith took to Brazil a quantity of 17D virus pre pared for human use in the Yellow Fever Laboratory in New York to test its efficacy under field conditions In September 1938 after a year s experi ence in the production and application (more than 59 000 vaccinations) of yellow fever vaccine made from the cultured 17D virus strain Smith Penna and Paoliello reported that there was now available a practicable safe method of large scale immunization against yellow fever

In summary it may be said that the chief landmarks in the conquest of yellow fever were (*a*) the work of the Reed commission in proving that yellow fever is carried by a mosquito and that the causative agent is ultra microscopic and filtrable (*b*) the discovery of a susceptible laboratory ani mal (*c*) the development of the mouse protection test (*d*) the development of successful techniques for eradication of the urban vector *A aegypti* (*e*) the discovery of the jungle cycle of yellow fever and its role in the epi demiology of the disease (*f*) the elaboration of a simple and practicable method of vaccination (*g*) the contribution to the techniques of virology and epidemiology in general

2 THE VIRUS

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THE MODERN phase of yellow fever research was initiated by the West Africa Yellow Fever Commission of The Rockefeller Foundation At the time this commission was formed, in 1925, the cause of yellow fever as it occurs in South America was almost universally believed to be the *Leptospira icteroides*, described by Noguchi The main object of the commission was to determine whether the disease diagnosed as yellow fever in Africa was identical with South American yellow fever The failure to find any evidence that leptospira was involved in cases of African yellow fever led the commission to seek another possible etiologic agent The main line of attack here was to search for a susceptible animal In an effort to find such an animal, Dr Henry Beeuwkes, director of the commission, imported animals from parts of the world distant from West Africa Among these animals were Indian rhesus and crown monkeys and Brazilian marmosets

The first shipment, consisting of nine crown monkeys, reached Accra in West Africa, in 1927 while an extensive epidemic of yellow fever was occurring there Inoculation of six of these monkeys with blood from yellow fever patients produced fever in four of them, followed by their collapse and death The macroscopic as well as the microscopic lesions in these animals bore a striking resemblance to those observed in fatal human cases Three attempts were made to transfer the disease to normal monkeys of the same species Of the three animals inoculated, two developed fever and recovered, while one showed no reaction No attempts were made to transmit the infection by means of *Aedes aegypti*

With the arrival of a new shipment of monkeys, consisting of *Macacus rhesus* and Brazilian marmosets, indubitable evidence was soon obtained that the rhesus monkey is susceptible to the virus of yellow fever The first strain of the virus to be established and transmitted in series in monkeys came from a patient named Asibi, who was suffering from a very mild and clinically almost undiagnosable attack of yellow fever This virus was easily transmitted from monkey to monkey as well as from man to monkey by the injection of blood taken early in the course of the disease It was also transmitted from monkey to monkey by the bite of *A aegypti* (Stokes Bauer, and Hudson, 1928*b*)

The susceptibility of rhesus monkeys to yellow fever was soon confirmed

by other workers in Africa and South America Thus Mathis Sellards and Laigret (1928) in Dakar were able to infect rhesus monkeys from a human yellow fever patient not only by blood inoculation but also by the bite of mosquitoes that had fed on the patient The strain of virus from this patient which is known in the literature as the French strain was shown to retain its viability in monkey liver while in the frozen state and was transported to England and to the United States In South America Aragao (1928) and Davis and Burke (1929) isolated various strains of yellow fever virus in rhesus monkeys

The finding of a readily susceptible animal opened up a productive field of research Thus Bauer and Mahaffy (1930*a*) showed that several species of African monkey were susceptible to the virus None of the animals succumbed to yellow fever infection but the virus persisted in their blood for a number of days and could be recovered again by the injection of the blood into the more susceptible rhesus monkeys Bauer (1928) first showed that mosquitoes other than *A aegypti* could under laboratory conditions transmit yellow fever virus from monkey to monkey These two findings namely that indigenous animals are susceptible to the virus and that several species of mosquito other than *A aegypti* can act as vectors suggested that the epidemiology of yellow fever might be more complex in pattern than was commonly believed at the time

Important early work on the virus following the discovery of a susceptible laboratory animal included the confirmation of the filtrability of the virus (Bauer and Mahaffy 1930*b*) the demonstration of antibodies in man and monkeys and the proof that the virus of yellow fever as it occurs in Africa is immunologically the same as that found in South America (Theiler and Sellards 1928 Sawyer Kitchen et al 1930)

In a search for additional susceptible laboratory animals Theiler (1930*b*) using the French strain discovered that the common white mouse was susceptible to an intracerebral but not to an intraperitoneal inoculation The disease produced in the mouse was an encephalomyelitis transmissible in series in mice with the virus localized in the nervous system This serial passage produced two modifications in the behavior of the virus Firstly it became progressively more virulent for the mouse the incubation period in successive passages becoming shorter and secondly it lost its pathogenicity for rhesus monkeys on extraneural inoculation These modifications of the virus led directly to two methods of vaccination of man against yellow fever one of which is still used This is the method employed by

the French authorities in West Africa and consists in vaccinating with mouse adapted strain The other method now discarded consisted in munizing man by the simultaneous inoculation of the mouse adapted vi and yellow fever immune serum

Of great importance to the study of the epidemiology and distribution yellow fever was the development of the use of mice in tests of immunit (Sawyer and Lloyd 1931) In fact the mouse has become indispensable in modern yellow fever research

Because the modification of the virulence of the virus induced by its serial passage in mice was not considered by American and British workers adequate for safe vaccination numerous efforts were made to find a means of producing a more marked attenuation This was finally achieved by the prolonged cultivation in vitro of virus of the Asibi strain (Lloyd Theiler and Ricci 1936 Theiler and Smith 1937*a* and *b*) This variant produced in tissue culture is known as the 17D strain and is still extensively used for human vaccination

In order to describe the different tissue affinities of the yellow fever virus workers in the field have established various terms The virus as it occurs in nature has affinities for cells derived from all three embryonal layers and is usually termed pantropic A strain of the virus is said to be viscerotropic when it has an affinity for the visceral organs of a susceptible animal that is organs other than the nervous system By viscerotropism is meant this affinity for extraneural tissue and the capacity of the virus to multiply and to produce lesions in the viscera The degree of virulence of the virus for the extraneural tissues is a measure of its viscerotropism Thus the marked viscerotropism of the Asibi strain for rhesus monkeys is made evident by two facts namely that it causes death from liver necrosis in approximately 95 per cent of the inoculated monkeys and that the disease it produces in the animals is manifested by a very high concentration of the virus in the circulating blood Since the liver is one of the chief organs affected the term hepatotropic is also in common use

Most if not all unmodified viruses also show neurotropic affinities that is affinities for the cells of the nervous system These can most readily be demonstrated by the intracerebral inoculation of mice which then develop an infection of the nervous system By continued serial passage in mouse brain the neurotropism is increased as manifested by a progressively shorter incubation period Such a virus is said to show marked neurotropism for the mouse It is said to be fixed when the incubation period re

constant and cannot be reduced on further passages By various means the two major affinities of yellow fever virus have been modified The most marked modification is that of the 17D strain of tissue culture virus which has become attenuated in both its viscerotropic and neurotropic affinities

PHYSICAL AND CHEMICAL PROPERTIES OF THE VIRUS

In considering the physical and chemical properties of yellow fever virus it must always be borne in mind that this virus remains active only in a diluent containing protein Bauer and Mahaffy (1930*b*) found that it readily becomes inactivated in 0 9 per cent sodium chloride solution Locke s solution Ringer s solution hormone broth or distilled water The deleterious action of these media on the virus is reduced if 10 per cent or more of normal rhesus serum is added

As a consequence of these findings it is routine in yellow fever work to use a diluent containing normal serum For most purposes normal human serum may be used The best diluent however has been found to be physiologic saline containing 10 per cent normal rhesus serum Many efforts have been made to discover a substitute for normal rhesus serum Dick and Taylor (1949) found that a diluent consisting of a solution of 0 2 per cent bovine albumin in buffered saline was satisfactory for many viruses including the virus of yellow fever The lability of yellow fever virus was the reason for the incorporation of normal human serum in the earlier vaccines

Like most viruses the virus of yellow fever is inactivated by heat The United States Army Yellow Fever Commission under the direction of Walter Reed (Reed and Carroll 1902) showed that in infective human serum it was completely inactivated at 55°C in 10 minutes Marchoux Salimbeni and Simond (1903) reported that 5 minutes exposure at this temperature was adequate for inactivation These pioneer experiments were of necessity limited as human volunteers had to be used With the availability of susceptible animals the monkey and the mouse the effect of heat on both the unmodified viscerotropic virus and the modified neurotropic virus was studied by Frobisher (1930) This author was able in the main to confirm the pioneer work He found that at 60°C both strains were readily inactivated in 10 minutes but that at 55°C they were sometimes inactivated and at other times were not Virus in the desiccated state was more heat stable than virus in fresh monkey serum or in suspensions

of infective mouse brain Thus the unmodified French strain in desiccated blood was not completely inactivated in 10 minutes at 60°C

Burruss and Hargett (1947) tested the heat stability of desiccated vaccines When the vaccines were maintained at 37°C an average of 90 per cent of the virus was lost in 2 weeks and 99 per cent in 8 weeks However some active virus was still present after 104 weeks When kept at a temperature of 80°C for 7 or 8 hours all of eight lots still contained sufficient virus for immunization It required 7 or 8 hours exposure to inactivate all the virus at 110 and 100°C These authors concluded that adequately desiccated vaccines after an exposure of several weeks to a tropical temperature may still contain enough virus for human immunization

FILTRATION

That the causative agent of yellow fever is capable of passing through bacteria proof filters was first shown by the Reed commission (Reed and Carroll 1902) This was confirmed by the French commission in Rio de Janeiro (Marchoux Salimbeni and Simond 1903) and by Rosenau Parker et al (1905) Because in all these filtration experiments human volunteers had to be used the observations were few in number

Following the discovery of the susceptibility of the rhesus monkey to the virus of yellow fever more extensive filtration studies were undertaken Stokes Bauer and Hudson (1928*b*) showed that the virus in the circulating blood of monkeys readily passed through Berkefeld filters of V and N grades and also through Seitz asbestos filters but not through Berkefeld W filters However the virus as it occurs in the mosquito was apparently not filtrable This latter observation was of significance for if true it would indicate that the virus in the invertebrate host differed in size or in some other properties from that in the vertebrate host and would suggest that some developmental cycle occurred

An extensive series of experiments designed to determine more fully the factors involved in filtration was reported by Bauer and Mahaffy (1930*b*) The results of their work showed that the virus both in the blood of infected monkeys and in infected mosquitoes passed through Berkefeld filters of all grades without a marked diminution of concentration and also through Chamberland L11 candles The finding that yellow fever virus is rapidly inactivated when diluted in saline explained the earlier unsuccessful filtration experiments with infected mosquitoes for in these experiments

the insects were ground up in a mortar with saline By the use of normal serum or a diluent containing 10 per cent normal serum it was readily shown that the virus in the mosquito was filtrable with ease Sawyer and Frobisher (1929) independently studied the filtrability of the virus as it exists in the mosquito and showed that in *A aegypti*, both in the infective stage and in the incubation period the virus is capable of passing through Berkefeld N filters when suspended in normal monkey serum

The finding that the virus of yellow fever is rapidly inactivated when diluted in physiologic saline solution must always be borne in mind when interpreting the earlier work on this disease The discovery that the addition of normal serum to a diluent was essential for accurate titration experiments was of extreme importance

The facts concerning the filtrability of yellow fever virus as it occurs in monkey serum were shown to apply to the neurotropic strain in brain tissue Here too it was found that the virus was rapidly inactivated when diluted in saline In order to obtain successful filtration a diluent containing normal serum had to be employed Under these conditions the neurotropic strain was readily filtrable through the usual laboratory filters

The ease with which yellow fever virus passed through the usual filters indicated that it was in all probability of small size Accurate determinations of the particle size had to await the perfection of methods for preparing membranes of uniform porosity This was achieved by Elford (1931) who made graded collodion membranes of uniform pore size Findlay and Broom (1933) using this method estimated the size of the French neurotropic strain to be between 18 and 27 mμ These figures are based on the experimental finding that the virus was able to pass through a membrane with average pore size of 55 mμ but was retained by one having an average pore size of 50 mμ A similar result was obtained when the unmodified French strain was used

More extensive filtration experiments through collodion membranes were reported by Bauer and Hughes (1935) The membranes used by these workers were prepared by the method of Elford with certain minor modifications (Bauer and Hughes 1934) In the main their results confirmed those of Findlay and Broom However possibly because of the use of a different diluent the virus was able to pass through a membrane of average pore size of 50 mμ and was retained by one having an average pore size of 15 mμ Elford has pointed out that membranes having pore diameters of from 10 to 100 mμ will allow only those particles to pass which are much

smaller than these figures By passing through graded series of filters colloidal particles with dimensions determined by other means Elford demonstrated that the average pore size must be from two to three times the particle diameter Applying these factors to the results of Bauer and Hughes the best estimate of the particle diameter of yellow fever virus is 17 to 25 mμ Identical results were obtained by Bauer and Hughes with the two strains under investigation Like Findlay and Broom they worked with the French neurotropic variant For their experiments with a viscerotropic strain they used the unmodified Asibi virus Both these strains are highly pathogenic for mice by intracerebal inoculation and consequently these animals were used to determine the infectivity of all filtrates The diluent chosen consisted of ascitic fluid hormone broth and phosphate buffer in distilled water The hormone broth was added because experience had shown that this facilitates the filtration of viruses (Ward and Tang 1929) The use of broth in all probability explains the slight discrepancy between the findings of Findlay and Broom and those of Bauer and Hughes

CENTRIFUGATION

An attempt to determine the particle size of yellow fever virus by ultracentrifugation was reported by Pickels and Bauer (1940) The air driven vacuum ultracentrifuge described by Bauer and Pickels (1937) and Pickels (1938) was used in these studies As a source of virus sera from monkeys infected with the Asibi strain were used The results of the ultrafiltration studies as well as the behavior of the virus in the high speed concentration centrifuge (Bauer and Pickels 1936 Hughes Pickels and Horsfall 1938) (Fig 18) gave information concerning the approximate size of the virus particles It was found that when infective monkey serum was run in the ultracentrifuge a faint absorption boundary was observed when the source of illumination was the 365 mμ line of mercury The boundary sedimented at the approximate rate predicted for yellow fever virus No evidence of such a boundary was found with either normal or immune monkey sera Furthermore such a boundary could be observed only in sera taken from the monkeys late in the disease From an extensive series of experiments the authors computed the size which was in approximate agreement with that found by ultrafiltration studies On the assumption that the virus particle was spherical and had a density of 1 33 gm per cc similar to that of most proteins Pickels and Bauer calculated the particle diameter to be

about 12 mμ Assuming the density to be 1 15 or 1 50 gm per cc the calculated diameter was 19 and 10 mμ respectively These computations of size are in approximate agreement with those obtained from filtration studies

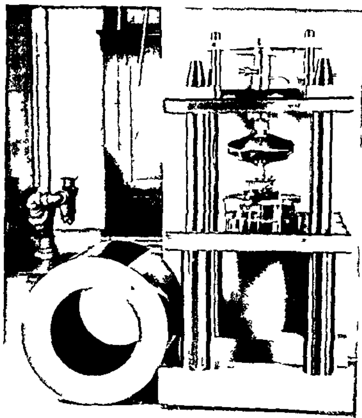


Fig 18 The ultracentrifuge used in recent studies on yellow fever

PRESERVATION

Methods which have been found satisfactory for the preservation of other viruses have also proved of value with yellow fever virus Like many other viruses this will maintain its viability while frozen Sellards transported virus of French strain from Dakar to England and America in the frozen state (Sellards and Hindle 1928) In the storage of virus for relatively short periods of time it is a common practice to keep infective material in the freezing chamber of an ordinary refrigerator

The storage cabinet devised by Horsfall (1940) in which the temperature

is maintained at −76°C by means of solid carbon dioxide has been found extremely satisfactory but the lusteroid tubes and metallic screw tops recommended by him cannot be used for yellow fever virus. Yellow fever virus maintained in these tubes rapidly becomes inactivated, probably due to entrance of carbon dioxide gas. Virus preparations sealed in glass ampules, however, maintain their activity unimpaired for a long period of time.

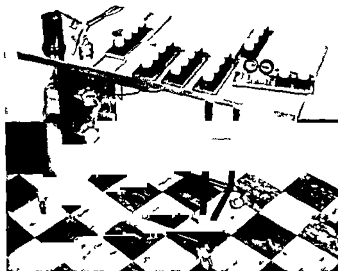


Fig 19 Apparatus for opening eggs under sterile conditions

In the large scale manufacture of yellow fever vaccine it was necessary to keep the infective chick embryo juice in bulk for bacterial sterility tests before further processing (Fig 19). Experience showed that infective chick embryo juice kept frozen at −18°C in a refrigerator rapidly lost its infectivity. This loss occurred whether or not normal serum was added to the chick juice before freezing. However, it was found that the activity of the virus could be maintained by keeping the infective juice at the temperature of dry ice, provided the bottles containing the material were tightly sealed with rubber stoppers.

The method of preservation that has been most extensively used consists of desiccating the virus containing material while it is in the frozen state (Sawyer, Lloyd and Kitchen, 1929) (Fig 20). At first desiccators of the Hempel improved type were employed using concentrated sulfuric acid to

absorb the water During drying the virus preparations were kept frozen by placing the desiccators in a bath of salt and ice The desiccated material was stored in the refrigerator in sealed glass tubes or ampules At times to make certain of complete dryness calcium chloride was placed above cotton plugs before the tubes were sealed in the flame This method proved to be highly efficient as is shown by the fact that a preparation of desiccated

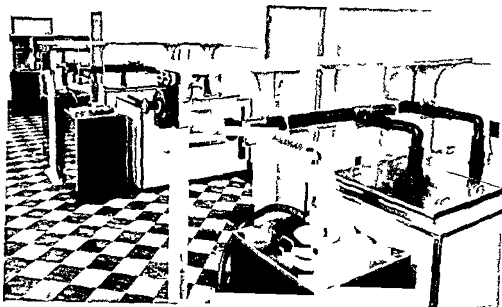


Fig 20 Laboratory for the large scale desiccation of vaccines The four units shown dry 20 liters of material within 24 hours

blood obtained from a monkey infected with the Asibi strain still contained active virus after 10 years storage in the refrigerator

With the development of methods of vaccination the need arose for large quantities of desiccated vaccine Through extensive work by Bauer and Pickels (1940) apparatus was devised which would accommodate large numbers of ampules of vaccine to be desiccated while in the frozen state The fluid vaccine in the ampules was rapidly frozen in a bath of alcohol containing solid carbon dioxide The ampules holding the frozen virus preparation were quickly placed on a large manifold connected with a vacuum pump (Fig 21) A cold trap served as a condenser This consisted essentially of a brass cylinder immersed to almost half its depth in alcohol and dry ice in a large thermos jar With the early models the manifolds were put in a refrigerator kept at a temperature of −18°C The mainte

nance of the material to be desiccated at this low temperature made the drying process relatively slow

Toward the end of the large scale wartime vaccine production a somewhat different apparatus was introduced Experience had shown that it was not necessary to keep the material to be desiccated at a low temperature Consequently in the new apparatus the ampules were maintained at room temperature The cold trap consisting as usual of alcohol and solid carbon dioxide was in the center of the manifold With the greater temperature difference between the material to be desiccated and the cold trap as well as the shorter distance traveled by the water molecules before their entrance into the trap the process of desiccation was markedly speeded up It could now be satisfactorily accomplished in 6 hours and the vaccines had excellent keeping qualities

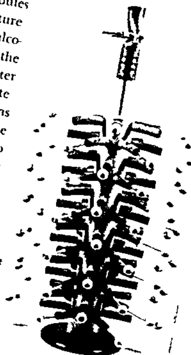


Fig 21 A manifold used in the preparation of yellow fever vaccines to freeze and dry vaccines capacity 72 ampules of vaccine Each ampule contains 2 cc of desiccated vaccine

It is a common experience that different batches of desiccated virus preparation vary in their keeping qualities when stored in the refrigerator In some the activity of the virus is maintained unaltered whereas in others there is a gradual loss of activity ox and Gard (1940) showed that the rate loss of activity of desiccated virus is proportional to the residual moisture the er the moisture content the better the ping qualities These authors also nd that a desiccated virus preparation keep its activity better when sealed in vacuum or in an atmosphere of gen than when sealed in an atmosphere of air rruss and Hargett (1947) as a result of numerous tests recommend esiccated vaccine be stored at 20 to −27°C Vaccine stored at 5 to showed considerable loss of virus activity during a storage period of

SUSCEPTIBILITY OF ANIMALS TO THE VIRUS

Since the discovery of the susceptibility of the monkey to yellow fever a great number of other animals have been tested, and many of these, from diverse divisions of the animal kingdom, have been found susceptible to the disease to a greater or lesser extent. With this plethora of observations a certain amount of selection must be made for the purposes of this discussion. The discovery of jungle yellow fever indicated clearly that wild animals play an important part in maintaining the virus in nature. However, the susceptibility of wild animals will not be discussed in this chapter except where the infection in them illustrates some particular property of the virus.

Here the main emphasis will be placed on the susceptibility of the common laboratory animals and on the types of infection produced in them. The primary concern will be the tissue affinities shown by the virus in different experimental hosts and the variation that can be produced by laboratory procedures. The virus of yellow fever, perhaps more than any other virus, can be modified by artificial means. The rhesus monkey has been chosen for major discussion, chiefly because the disease produced in this animal resembles in many ways yellow fever as seen in man and also because monkeys are known to play a part in the epidemiology of yellow fever. The response of the rhesus monkey to several strains of virus of widely differing tissue affinities will receive considerable attention. The strains of virus selected for discussion are the Asibi, the French neurotropic, and the 17D. The Asibi strain was chosen because it was isolated first and exhibits viscerotropism to a marked degree. French neurotropic and 17D are modified strains which are being used extensively for human vaccination, and for this reason the infection produced by them in experimental animals will be considered in some detail.

SUSCEPTIBILITY OF RHESUS MONKEYS TO *VISCEROTROPIC STRAINS*

The first evidence of the susceptibility of monkeys to the virus of yellow fever was obtained by Stokes, Bauer, and Hudson (1928*b*), who produced infection and death in the Indian crown monkey *Macacus sinicus*, by inoculation of blood from a human yellow fever patient. Their attempts to trans

mit the infection to other monkeys of the same species were unsuccessful From these experiments they concluded that crown monkeys were susceptible to the virus of yellow fever only to a moderate degree They had greater success with the common Indian monkey *M rhesus* They could readily transmit yellow fever from man to this species of monkey They found that the infection was easily transmitted from monkey to monkey by the injection of citrated blood taken early in the course of the disease and they had no difficulty in transmitting the infection to monkeys by the bite of *A aegypti* The virus with which all this pioneer work was done was obtained from the patient named Asibi who was suffering from a mild attack of the disease In spite of the mildness of his attack the virus isolated from him was extremely virulent Infection induced in rhesus monkeys with this strain is usually very acute and almost invariably ends in death After an incubation period of 2 or 3 days the animal develops a fever and death usually occurs on about the 5th day In a typical fatal infection virus is present in the blood in very high concentration Thus Bauer and Mahaffy (1930*a*) found that blood from an infected monkey in amounts as small as one hundred millionth of 1 0 cc was capable of producing infection in other monkeys Bauer (1931*a*) in titration experiments conducted later showed that at times there is sufficient virus to produce a fatal infection in 1 0 cc of blood when diluted 1 1 000 000 000 It is of interest that by the use of mice and the intracerebral route of inoculation titers of the same order of magnitude are obtained Thus it is by no means unusual to produce fatal encephalitis in mice with infective monkey serum inoculated in 0 03 cc amounts in dilutions of 1 10 000 000 and sometimes even 1 100 000 000

The incubation period in monkeys inoculated with small quantities of virus tends on the average to be longer than that observed in monkeys inoculated with large doses Thus Bauer (1931*a*) has reported incubation periods of up to 19 days in monkeys inoculated with very small doses of the Asibi strain The disease produced in monkeys by minimal amounts of virus even after a long incubation period is just as fulminating as that occurring in animals receiving large infective doses In a typical infection with a short incubation period and death on the 5th or 6th day virus is present in the blood in high concentration throughout the disease Should the disease be unusually prolonged however there is a tendency for the virus to disappear Bauer (1931*a*) was able to show that in such instances antibodies are present in the blood at the time of death Thus in protection

tests with the serum of monkeys taken at death antibodies were not demonstrable in any of nine animals dying on the 4th and 5th days but were present in all of eight monkeys dying on the 7th to the 9th day The presence of antibodies at death depended *on the duration of the disease and not* on the incubation period Thus of five monkeys dying from a fulminating infection on the 8th to the 18th day following inoculation with small amounts of virus none had demonstrable antibodies in the blood at the time of death

The long incubation periods recorded by Bauer have rarely been observed in the Yellow Fever Laboratory in New York Monkeys inoculated with from 10 to 100 LD_{50}[1] for mice have invariably developed a fulminating infection and have died on the 4th to the 8th day

While the majority of viruses isolated in Africa show as great pathogenicity for rhesus monkeys as the Asibi virus a somewhat different picture is produced by most of the strains studied in South America which are relatively avirulent For example Davis and Burke (1929) had great difficulty *in attempts to isolate virus from South American yellow fever patients* concluding that it takes much care patience and an abundance of monkeys to build up and maintain a high degree of virulence The Brazilian F W strain was studied by Sawyer Kitchen et al (1930) who observed death in only two of 20 inoculated monkeys Laemmert (1944) gave *some figures* concerning the death rate in rhesus monkeys inoculated with strains of jungle yellow fever isolated in Brazil Colombia and Bolivia The mortality varied from 100 per cent with a Bolivian strain to 9 per cent with a Brazilian strain However the Bolivian figure is based on only four animals On occasion relatively avirulent strains have been observed in Africa Hudson stated that in the laboratory of the West Africa Yellow Fever Commission a strain H P isolated from a fatal human case killed only one third of inoculated animals (quoted by Sawyer Kitchen et al 1930) *In general however rhesus monkeys seem to be equally susceptible* to African and South American strains but the disease produced by most South American strains is more often nonfatal The severity of the disease in humans from whom the virus is isolated apparently is not related to the virulence of the virus for rhesus monkeys Rhesus monkeys inoculated with relatively avirulent strains as a rule show no symptoms of infection Usually after an incubation period that is quite variable a fever develops but the occurrence of fever is not a good indication of the stage of infection

[1] *An LD_{50} is the dose that is lethal to 50 per cent of the test animal*

By the use of mice the exact course of the blood infection can be readily followed By this means it has been found that the fever often occurs after the virus has disappeared from the blood

Bauer and Hudson (1938*a* and *b*) showed definitely that yellow fever virus can penetrate the intact rhesus monkey skin Attempts to infect monkeys by mouth or by placing the virus in the conjunctival sac were unsuccessful Findlay and MacCallum (1939*a*) were able to produce infection readily in rhesus monkeys by the administration of the virus by means of a catheter passed into the stomach They succeeded in producing infection with unmodified viscerotropic virus as well as with strains modified by either mouse brain passage or tissue culture In each instance infection was proved by the demonstration of virus in the circulation and in those animals that did not die of visceral yellow fever by the demonstration of the development of antibodies Similar results were obtained with the same technique in four African monkeys of the species *Cercopithecus aethiops* Numerous attempts were made by Findlay and MacCallum to determine whether virus was excreted in the feces or urine of infected rhesus monkeys all with negative results

Monkeys inoculated intracerebrally with highly viscerotropic strains of yellow fever virus such as the Asibi or the French die as a rule of acute visceral yellow fever Microscopic examination of the brains of such animals shows no or at most minimal signs of encephalitis However Findlay and Stern (1935) and Penna (1936) found that if an intraperitoneal or subcutaneous inoculation of immune serum is given slightly before or at the same time that virus is inoculated intracerebrally monkeys die of encephalitis Pathologic examination of these animals reveals marked encephalitis but no or slight lesions in the liver Findlay and Stern noted that if the immune serum is given after the intracerebral injection of virus the animals die of visceral yellow fever While Penna used only the Asibi strain in his experiments Findlay and Stern used both the Asibi and the French strains Both workers were able to passage the virus in monkeys by intracerebral inoculation of brain material Findlay and Stern found that at the time of death virus was present only in the brain not in the blood or liver of those monkeys that received immune serum before the intracerebral inoculation of virus Penna in his experiments found the virus widely distributed throughout the organism that is in the brain the sciatic and median nerves the spleen the adrenal inguinal and mesenteric lymph glands and the testicle but not in the blood serum This distribution in

dicates that the virus produced not only an encephalitis but a systemic infection as well. It is probable that the systemic infection was of a modified type such as will be described in the section dealing with the course of infection produced by the French neurotropic virus on extraneural inoculation into passively immunized rhesus monkeys. In the case of Penna's monkeys, the immune serum was sufficient to protect the liver from massive infection and thus prevent death from hepatic necrosis, but it did not prevent infection of other organs, such as the spleen or the lymph glands. Findlay and Stern presented some evidence that after three serial brain passages in monkeys the virus became more neurotropic for mice.

Penna studied the effect of serial brain to brain passage of the Asibi virus in monkeys receiving immune serum intraperitoneally. To determine whether any marked change was induced in the passage virus, monkeys were inoculated intracerebrally at intervals, without the simultaneous injection of immune serum. Two parallel series of passages were maintained. In the first, the virus was propagated by intracerebral inoculations in passively immunized animals for 40 passages without any evidence of modification of its viscerotropism. Animals inoculated into the brain with virus representing the 3d 7th, 15th, and 40th passages, without the simultaneous inoculation of immune serum, died of typical visceral yellow fever. In the second series, a modification of this virus was first observed in its 35th passage. Monkeys inoculated intracerebrally with the 35th, 36th, and 10th passage virus all died of typical yellow fever encephalitis without evidence of visceral lesions, even though no immune serum had been given. This series was continued in monkeys by brain to brain transfer without immune serum for 12 passages. The results of these passages showed that as yet the virus was still somewhat viscerotropic, as three animals died of acute visceral yellow fever. The serial passage of the virus was consequently continued in animals receiving immune serum at the time of inoculation. The serial transfer of the virus in passively immunized monkeys was continued to the 85th passage, when it was tested by intracerebral inoculation of monkeys without immune serum. All of seven monkeys inoculated with this virus died of encephalitis without evidence of visceral involvement, and no lesions of yellow fever were found in sections of the liver. Before the termination of the series, the pathogenicity of the passage virus was tested by *subcutaneous or intraperitoneal inoculation. Of the 11 monkeys so inocu*lated one was killed for study and the rest lived and were later proved to have been immunized against yellow fever.

The results of these experiments indicate clearly that an unmodified highly viscerotropic yellow fever virus is essentially neurotropic for monkeys. By continuous passage in monkey brain the virus loses its viscerotropism to a considerable extent so that, unlike the original virus, it is no longer able to kill the animal by producing visceral lesions. It now acts in monkeys very much like a virus modified by prolonged passage in mouse brains. No evidence is available concerning the pathogenicity for mice of the Asibi strain modified by intracerebral passage in passively immunized monkeys.

It is of interest that monkeys inoculated intracerebrally with unmodified virus possessing low viscerotropic affinities usually live. However, such monkeys react to an intracerebral inoculation with a more marked febrile reaction than monkeys inoculated by extraneural routes with the same strains, suggesting that they have had a nonfatal encephalitis.

In several yellow fever research laboratories accidental infection of rhesus monkeys has been observed. On a number of occasions uninoculated rhesus monkeys have developed antibodies to yellow fever. These immunizations have been observed to occur not only in monkeys kept in the same room as animals under experimentation but also in rooms where only normal uninoculated animals were kept. Conclusive evidence of accidental yellow fever infection was the death, from typical visceral yellow fever, of several uninoculated monkeys. Virus isolated from these animals behaved in all respects like a typical viscerotropic strain and was specifically neutralized by yellow fever immune serum. Findlay and MacCallum (1939) reported the occurrence of fatal accidental yellow fever infection in two monkeys. The first of these animals to die had been in a room where no other monkey infected with yellow fever had been kept for nearly three months, whereas the second animal was in a room in which no other infected monkeys had been kept for nearly six months.

No evidence is available as to how these accidental infections occur. Transmission by mosquitoes can be definitely excluded. The possibility that infection is by mouth is suggested by the fact that Findlay and MacCallum (1939c) infected monkeys by administering yellow fever virus by catheter into the stomach.

While it is clear that the fatal accidental infections were caused by a virulent viscerotropic strain, the more numerous instances of nonfatal accidental infections are probably due to a neurotropic virus. In the Yellow Fever Laboratory in New York conclusive evidence that accidental infec

tions with a neurotropic virus can occur was obtained by the isolation of a typical neurotropic strain from the brain of a monkey that had been kept in the normal monkey room The animal showed clinical signs of encephalitis This accidental infection occurred at a time when a great amount of work with the French neurotropic virus was in progress and when several normal monkeys developed specific antibodies

COURSE OF INFECTION IN RHESUS MONKEYS INOCULATED WITH VISCEROTROPIC STRAINS

In yellow fever studies the serum of an infected monkey is generally used as a source of the virus because of the high titer of the virus in the blood when this is taken at the appropriate time and because of the ease of handling the serum Occasionally however other organs particularly the liver have been used While it is known that the liver of an infected monkey is usually infectious no information is available as to the relative concentration of the virus in the various organs It was assumed that the lesions observed in the visceral organs at death were due to the action of the virus Hence arose the term viscerotropism Attempts to titrate the virus content of organs in monkeys infected with the Asibi strain were always complicated by the fact that the blood contained virus in high concentration and consequently there was no way of knowing how much of the virus found in a tissue was due to its presence in the blood Titrations in mice of serum and liver of monkeys killed during the course of infection with the Asibi strain usually gave results which indicated that there was a higher concentration of virus in the serum than in the liver That this is in all probability not a true indication of the relative concentration is shown by the fact that saline extracts of normal monkey liver have a deleterious effect on the virus Consequently extracts of infective monkey livers would probably give far too low a virus titer The nature of the deleterious substance is not known though it has been demonstrated that the bile of normal monkeys even in a hundredfold dilution has virucidal properties In order to obtain some idea of the actual concentration of virus in the liver of an infected monkey eliminating as far as possible the virus in the blood a monkey inoculated 3 days previously with the Asibi virus was bled to death and perfused with saline to render the organs as free of blood as possible The titer of virus in the serum at the time the monkey was killed was 1 1 000 000 As a control of the efficiency of the perfusion some of the wash fluid flowing out of the animal at the end

of this process was taken and an equal amount of normal serum was imme diately added The titer of virus in this terminal portion of wash fluid was 1 200 clearly indicating that perfusion of the monkey had been very effi cient in removing most of the virus from the circulation On the assumption that 10 per cent of each organ consisted of perfusion fluid the titer of virus in any organ due to the contained fluid would be 1 20 A titer higher than 1 20 would indicate the presence of virus over and above the amount due to the virus in the perfusion fluid The organs tested and the titers of virus in each beginning with the highest were as follows liver 1 150 000 bone marrow 1 3 700 spleen 1 1 000 kidney 1 80 adrenal gland 1 70 mesen teric lymph glands 1 10 and brain a trace of virus By far the largest con centration occurred in the liver followed by that in the bone marrow and spleen The finding of the highest concentration in the liver is not surpris ing as this organ shows the greatest pathologic changes

In an attempt to get more information on the course of infection and the tissue affinities of an unmodified virus six monkeys were inoculated with the F W strain This virus is relatively avirulent for the rhesus monkey seldom causing death Approximately 100 LD $_{50}$ as determined in mice were inoculated intradermally on the ventral aspect of the right thigh just below the inguinal region One infected animal was killed on each of the following days after inoculation 1st 2d 3d 5th 7th and 9th the titer of virus in various organs was then determined Organs tested for virus were the skin at the site of inoculation the local lymph glands retroperitoneal and mesen teric lymph glands liver spleen kidney adrenal gland and bone marrow An attempt was made to determine the amount of virus in most of the organs by the inoculation of mice with serial decimal dilutions of these tissues Daily examination for circulating virus showed that the first three monkeys were killed before the second two during and the third several days after the blood stream invasion

No virus was recovered from the monkeys killed 1 and 2 days after inocu lation not even from the skin at the site of injection or in the local lymph nodes In the local lymph glands of the monkey killed on the 3d day only a trace of virus was *noted and the blood and all other organs were negative* In the animal killed on the 5th day at a time when the virus in the blood had a titer of 1 50 virus was present in all the organs tested the maximum quantity being in the mesenteric lymph node (1 3 200) The titer of virus in other organs were bone marrow 1 760 adrenal gland 1 130 right inguinal lymph node 1 200 liver 1 160 spleen 1 135 and kidney 1 10

The next monkey in the series killed 7 days after inoculation, at a time when virus had almost disappeared from the circulation, showed approximately the same quantity of virus in the bone marrow (1:720) and the adrenal gland (1:500), but considerably more in the liver (1:150). The titers of virus in the remaining organs were: spleen, 1:60, kidney, 1:30, mesenteric lymph glands, 1:32, and right inguinal lymph nodes 1:17. The last animal killed 3 days after the conclusion of blood stream invasion and at a time when antibodies could be demonstrated in the serum, had traces of virus in the local lymph glands and in the liver and spleen. As will be shown later, the sequence of events in monkeys infected with the relatively avirulent unmodified F.W. strain is very much like that observed in monkeys infected with the French neurotropic virus. However, there appeared to be a longer incubation period with the F.W. strain, as no virus was recovered from the local lymph glands until the 3d day after infection. Furthermore, the quantity of virus in the local lymph glands never reached the amount that is seen in infection with the French neurotropic strain. This may be a purely chance phenomenon, as the titer of virus in the mesenteric lymph glands of the animal infected with the F.W. strain was just as high as the highest titer obtained in the local lymph glands of the monkey infected with the French neurotropic strain. The results show that an unmodified strain of virus, such as the F.W., has a predilection for the liver, bone marrow, adrenal glands, and lymph glands.

The presence of virus in an organ does not necessarily indicate that it has multiplied in that organ. The concentration of virus may be caused by the accumulation due to the clearing mechanism of the reticuloendothelial cells. Thus, the virus found in the liver may be due to either multiplication in the hepatic cells or accumulation in the Kupffer cells. It seems probable that the highly virulent Asibi virus enters and multiplies in the parenchymatous cells of the liver. The indications are that the more virulent the strain, the more marked the hepatic necrosis and the higher the titer of virus in this organ. There is also very good evidence that yellow fever virus multiplies in lymphoid tissue. Relatively high concentrations in lymph glands as observed in these experiments with unmodified strains have also been observed in monkeys inoculated with the French neurotropic and 17D strains. Pathologic studies support these findings. Klotz and Belt (1930*b*) described definite changes in the lymph glands as well as in the lymphoid centers of the spleen in persons dead of yellow fever. Stokes, Bauer, and Hudson (1928*b*) observed pathologic changes in the spleen and lymph nodes of

monkeys that had died as a result of infection with the Asibi strain lesions in both these organs consisted of necrosis of the lymph nodules

Supporting evidence that yellow fever virus may also infect and cause lesions in the adrenal glands is the observation that in monkeys dead of yellow fever the cells of the zona fasciculata may be necrotic. Pathologic evidence that the bone marrow is involved was found by Berry and Kitchen (1931) who reported degenerative changes in the immature cells of the granulocytic series of monkeys infected with yellow fever.

Although the pathologic evidence presented is in favor of the hypothesis that virus multiplies in the organs in which the highest concentration is found all these organs are also rich in cells belonging to the reticuloendothelial system and it is clear that some of the virus could quite legitimately be considered as an accumulation due to the clearing mechanism. It was accordingly of interest to investigate the clearing mechanism in a normal monkey. An attempt to do this using essentially the technique that has proved so successful with bacterial infections was made with the F W strain of virus. Three monkeys were inoculated intravenously with infective serum estimated to contain 125 000 I D for mice. In the first of these animals the presence and amount of virus in the blood was determined at frequent intervals after inoculation. The results of the titrations showed that the clearing mechanism was highly efficient. The titer of virus in the blood 2 minutes after inoculation was 1 10. One hour afterwards it was 1 7. Two and 3 hours after injection virus was present in small amounts only one or two mice becoming infected when inoculated with the undiluted serum. Serum obtained after 4 and 5 hours contained no demonstrable virus. This monkey was killed 5 hours after infection and extracts of various organs were inoculated into mice to test virus content. The titer of virus in the blood of the second monkey of this series was determined on three different occasions after inoculation. After 2 minutes the titer was 1 20 at 5 hours there was no virus at 21 hours the titer was 1 32 and at that time the animal was killed and the virus content of the organs was tested. A third animal had a virus titer in the blood of 1 2 000 when killed 18 hours after infection.

In the first animal killed 5 hours after inoculation no virus was recovered from any of the organs. It appeared that the relatively small quantity of virus inoculated had been completely destroyed. In the second monkey killed 21 hours after infection at a time when virus had reappeared in the blood virus was recovered only from the liver in which the titer was 1 2[illegible]

In the third animal killed 2 days after inoculation and in which the titer in the blood was 1 2 000 virus was demonstrated in the spleen kidney bone marrow and liver Only in the liver however, was it present in any amount showing a titer of 1 2 700

The results of this experiment demonstrate the efficiency of the clearing mechanism of normal monkeys for yellow fever virus but give no definite information whatsoever as to what organs play a part in this mechanism The affinity of an unmodified virus for the liver is again evident and its rapid multiplication in this organ is striking

SUSCEPTIBILITY OF RHESUS MONKEYS TO A NEUROTROPIC STRAIN

The reaction of monkeys to an inoculation of neurotropic yellow fever virus differs somewhat from that seen following the inoculation of an unmodified virus By neurotropic yellow fever virus is meant a strain which has been maintained for a prolonged period of time by serial brain to-brain passage *in* mice

Of the numerous strains of yellow fever virus which have been passaged in this manner only the French strain has been studied extensively This strain was the first to be modified by mouse passage and it has come into widespread use not only for protection tests in mice but also for large scale vaccination of man

The modified or neurotropic French strain differs from the unmodified one in that it is more virulent for mice on intracerebral inoculation and it has lost to a large extent its pathogenicity for rhesus monkeys on extraneural inoculation Furthermore *A aegypti* can only with difficulty be infected with this strain (Davis Lloyd and Frobisher 1932)

Monkeys inoculated subcutaneously with the French neurotropic virus *may or may not have a febrile reaction* and usually *survive* In some animals however fatal encephalitis develops Irrespective of the outcome or the amount of the infecting dose virus can be demonstrated in the circulating blood The incubation period that is the time from inoculation to the first appearance of virus in the blood depends on the quantity of virus in the inoculum With a very large infecting dose virus may be present only on the 2 days following inoculation *On the other hand* when the animal receives a minimal infecting dose there may be an incubation period of several days before it appears in the blood (Theiler and Hughes 1935)

The Virus

When small quantities of virus are inoculated the time during whic can be demonstrated in the blood tends to be prolonged There can be doubt that after the extraneural inoculation of a neurotropic strain a temic infection takes place which differs from that produced by an unmo fied virus chiefly in that the titer in the blood is usually lower The exte of multiplication of the French neurotropic strain in monkeys followi the inoculation of extremely small amounts of the virus is shown graphicall by an example from some experiments by Theiler and Whitman (1935a) These authors inoculated a monkey with a dose of the virus which repre sented 2 LD$_{50}$ for mice and they determined the amount of virus in the circulation on alternate days commencing on the 1st day after inoculation The maximum quantity was found on the 5th day when it was present in a titer of 1 3 000 but virus was demonstrated in the blood though in smaller amounts on the 3d and 7th days as well This observation shows that the French neurotropic strain has the power of very rapid multiplica tion in rhesus monkeys when administered by extraneural routes

The observation that the prolonged passage of the French virus in mouse brain produced a marked diminution of its viscerotropic affinity and as a consequence made its use feasible for human vaccination raised the question of the stability of the virus If it could be readily reconverted to its original virulence its use for vaccination was potentially dangerous

Lloyd and Penna (1933a) made an exhaustive study of the pathogenicity of the French neurotropic virus for rhesus monkeys Their attempts to maintain the virus in monkeys in its 110th mouse brain passage by periph eral inoculation of blood from the preceding animal caused the loss of the virus This was no doubt due to the fact that these workers bled the animal for passage purposes at the time of fever It was later shown that pyrexia in monkeys inoculated with the French neurotropic strain often occurs after the blood stream infection has run its course The loss of the virus consequently was to be expected

In a similar type of study Theiler found no difficulty in maintain ing the French neurotropic strain through 20 monkey passages using the 102d mouse brain passage to initiate the series Passage was made by intra peritoneal inoculation of blood obtained on the 3d day after injection Five of the animals died death in each case being due to encephalitis There was no evidence of any increase in the virulence of the virus for monkeys The pathogenicity of the virus isolated from the 2d and 20th monkey pas sages was tested in mice by serial passages Both mouse passage series

identical indicating that the prolonged maintenance of the virus in rhesus monkeys had not led to any demonstrable change in its neurotropic affinity It acted throughout the experiment as a fixed neurotropic virus

Bates and Roca Garcia (1946c) maintained a fixed neurotropic virus in saimiri monkeys for 22 serial passages Using the French strain in its 250th mouse brain passage to initiate the series they inoculated monkeys intramuscularly with a tenfold dilution of the 4th day serum of the preceding animal Eleven of the 22 monkeys died In no case was there a well-defined febrile reaction Examination of the livers of the animals that died in no instance showed pathologic lesions typical of yellow fever although stomach hemorrhages were found in six of the animals No examination was made of the brain to determine whether encephalitis had occurred The cause of death in these animals consequently was not determined Assuming that death was due to yellow fever it appeared as though the virus became less virulent for the monkeys with passage since eight of the first nine infections were fatal whereas only three of the following 13 were fatal With each passage the titer of virus in the serum used for inoculation was determined There was no indication of either increase or decrease of circulating virus in successive passages nor did the virus show any demonstrable change in behavior when inoculated intracerebrally into mice Attempts by Bates and Roca Garcia to infect haemagogus mosquitoes failed completely when they allowed these mosquitoes to feed on monkeys at intervals throughout the passage series No evidence consequently was obtained in this passage series that the French neurotropic virus was altered in any way

Following the inoculation of neurotropic virus by extraneural routes rhesus monkeys occasionally show an infection of the central nervous system This has been observed when the virus has been inoculated subcutaneously intraperitoneally by scarification of the skin (Theiler and Hughes 1935) by intranasal insufflation (Findlay and Clarke 1935a) and by the bite of infected aegypti mosquitoes (Davis Lloyd and Frobisher 1932) In all cases that have been adequately studied irrespective of the route of inoculation encephalitis has developed after the mild systemic infection has run its course At the time of the development of encephalitis no virus can as a rule be demonstrated in the blood However antibodies are present The distribution of the virus in monkeys in which encephalitis develops is essentially the same as the distribution in animals inoculated directly into the brain that is the virus is confined almost entirely to nervous tissue

The incidence of encephalitis in monkeys inoculated by extraneural

routes is highest following intranasal instillation In all of six rhesus m[on]keys inoculated by this method Findlay and Clarke (1935*a*) found evide[nce] of infection of the central nervous system when the animals were killed [...] to 16 days after inoculation Theiler and Smith (1937*b*) summarizing th[eir] experiences in the Yellow Fever Laboratory in New York up to that time reported that after subcutaneous or intraperitoneal inoculation or the appli[-] cation of the virus onto the scarified skin an approximate incidence of 30 per cent of fatal encephalitis had been observed Encephalitis has been noted after the inoculation of very large as well as extremely small doses of virus It is the impression of workers in this field that encephalitis is more likely to follow infection with extremely small amounts of virus

In the work thus far discussed serial passage of a neurotropic strain of yellow fever virus by parenteral inoculation into monkeys had not led to any discernible change in the virus However Findlay and Clarke (1935*b*) reported experiments in which on two occasions a neurotropic strain was apparently reconverted into a viscerotropic one The French neurotropic strain after 182 and 212 mouse brain passages was used to initiate two series of liver to liver passages in monkeys The animals were killed after 4 to 7 days and transfers were made using a suspension in saline of the liver of the sacrificed animal Microscopic examination of the livers of all the animals showed lesions of varying extent In the first serial passage the 12th monkey died in 5 days The virus recovered from the liver and blood of this animal was inoculated subcutaneously into other monkeys which died with visceral lesions typical of yellow fever This reconverted virus was transmitted by parenteral inoculation in series through six passages in 13 monkeys of which 10 died or were killed when moribund in from 4 to 6 days while three recovered The reconverted virus behaved in rhesus monkeys mice and hedgehogs like a typical viscerotropic one

Essentially similar results were obtained in a second series of intrahepatic passages In mice the behavior of the reconverted strain resembled that of a viscerotropic one that is the incubation period was long By serial passage in mice this period became shorter and after 25 mouse brain passages the virus behaved like a fixed neurotropic one not only in mice but also in monkeys In the latter animal this mouse passaged virus on intracerebral inoculation produced encephalitis but no visceral lesions

The results of the serial passage of a neurotropic virus through 20 mon[k]eys by blood inoculation have been discussed above Furthermore it has [b]een shown as will be described later that this strain has an affinity for

liver cells Virus by whatever route inoculated reaches the liver by way of the blood stream Serial passage by intraperitoneal inoculation with blood from the previous animal must include the passage of virus derived from the liver It is doubtful if an intrahepatic inoculation leads to the infection of the liver to a much greater extent than inoculation by some other parenteral route In an attempt to repeat the observations of Findlay and Clarke Theiler and his associates made eight serial intrahepatic passages in rhesus monkeys The French neurotropic strain after 402 mouse passages was used to infect the first monkey in the series Liver from the second monkey inoculated intrahepatically was used to initiate a parallel series in which the passage was made by subcutaneous inoculation of liver extract In neither series after eight and six passages respectively was any modification of the virus observed No lesions were produced in the liver and the virus throughout the experiment behaved in mice like a fixed neurotropic strain It is possible that the difference between these results and those of Findlay and Clarke is due to the fact that Theiler and his co workers used the French virus after 402 mouse passages whereas Findlay and Clarke initiated their experiments after only 182 to 212 passages had been made Furthermore it is significant that the virus used by the latter authors to initiate their series of experiments produced lesions in the liver of the first monkey inoculated

The development of encephalitis in monkeys following the extraneural inoculation of the French neurotropic strain was considered by American and British workers as an indication that this virus was too neurotropic to be used for human vaccination without the simultaneous administration of immune serum

The observation that the French virus when modified by brain to brain passage in mice develops marked neurotropism for rhesus monkeys was first made by Sellards (1931) Lloyd and Penna (1933*a*) reported on extensive studies of the neurotropic properties of virus adapted to mouse brain when this was introduced into the nervous tissue of rhesus monkeys These authors observed that a fatal encephalitis could be readily produced by the intracerebral or intraspinal inoculation of this virus It was maintained with ease by brain to brain passage and could be transferred through 10 passages in duplicate series Each monkey was inoculated into the frontal lobe with a Berkefeld N filtrate of the brain of the preceding animal Twenty two of 23 rhesus monkeys inoculated died of encephalitis The incubation period that is the period from inoculation to onset of fever varied from

2 to 6 days, with an average of 4.2 days. The interval between the onset of fever and death varied from 1.5 days to 4.5 days, with an average of 2.9 days. The principal symptoms were all referable to the nervous system and included muscular tremors, ataxia, extensor palsies of the wrist, paresis and paralysis of different muscle groups, nystagmus, convulsions, salivation, and sudden acute pulmonary edema. In most instances, in monkeys inoculated intracerebrally with the neurotropic strain, the virus could be recovered from the blood stream at the beginning of fever. However, at death the blood was invariably virus free.

The distribution of the virus at death was determined in another series of monkeys infected by intraspinal inoculation. Each animal was inoculated with the brain filtrate of the preceding animal. In this series the average length of both the incubation period and the period of illness was greater than in monkeys inoculated intracerebrally. At the time of death virus was isolated from all portions of the nervous system, including brain, spinal cord, sciatic nerve, radial nerve, ulnar nerve, and retina. No virus was demonstrated in the blood, liver, kidney, spleen, or spinal fluid; however, it was found in the adrenal, parotid, submaxillary, and sublingual salivary glands. The distribution of the virus at death was essentially the same as that observed in animals infected with rabies virus, suggesting that the dissemination occurred centripetally along nerve pathways. It is probable that monkeys inoculated via the nervous system undergo a mild systemic infection such as that observed following the extraneural inoculation of virus in addition to the infection of the nervous system. The systemic infection, a manifestation of which is the presence of virus in the circulation, runs a rapid course and has subsided by the time symptoms referable to the nervous system appear.

Lloyd and Penna (1933a) noted no change induced in the virus as a result of the serial passage in the nervous system of monkeys. The virus at the beginning and at the end of their two serial passages acted in mice like a fixed neurotropic strain. There was no indication that its prolonged passage in monkeys rendered it more pathogenic for these animals.

COURSE OF INFECTION IN RHESUS MONKEYS INOCULATED WITH A NEUROTROPIC STRAIN

It is apparent that when a neurotropic strain of yellow fever virus is inoculated by extraneural routes it produces a systemic infection analogous to but

milder than that produced by the unmodified viscerotropic strain Monkeys killed during an infection of this kind *show no obvious microscopic lesions* such as are present in the liver kidney spleen or stomach of monkeys infected with a virulent viscerotropic strain No information is available in the literature as to the site of multiplication of the neurotropic virus during a systemic infection

The results of some experiments performed at the Yellow Fever Laboratory in New York with a view to gaining light on this point are reported here in summary form for the first time When these experiments were undertaken the French neurotropic virus had already been used for some time for human vaccination For the study of the spread and multiplication of the virus monkeys were given an intracutaneous *injection* of a small quantity of it The injections were made into the shaved skin on the ventral aspect of the right thigh approximately one inch below the inguinal region On the day following inoculation and on every 2d day thereafter each monkey still alive was bled from the heart and the serum thus obtained was inoculated intracerebrally into mice to test for virus in the circulation Individual monkeys were killed by bleeding them to death at intervals after *inoculation portions of various organs were removed weighed ground up* in mortars to make a 10 per cent suspension and lightly centrifuged and some of the supernatant fluid was injected intracerebrally into mice The results of the tests for circulating virus showed that one animal had been killed before two during and two after blood stream invasion The results of the infectivity tests with the organs from the monkeys killed 1 day after inoculation and before blood stream invasion showed that virus was present only *in the skin at the site of inoculation and in the local lymph glands*

In the second monkey killed on the 3d day virus was found in the skin the local lymph glands the serum the spleen the liver and the mesenteric lymph nodes but only in the local lymph glands was it present in sufficient amounts to suggest that multiplication had occurred The virus present in the other organs could be reasonably explained by the virus content of the blood

The serum of the third monkey *in the series killed on the 5th day* was moderately infective as indicated by the fact that five out of six mice died of yellow fever encephalitis when inoculated with a 1 10 dilution of it but all five mice inoculated with a 1 100 dilution lived With the serum containing so much virus it was to be expected that all the organs would be infective This was indeed the case However the infectivity of most of them

was tested in two dilutions 1 10 and 1 100 All deaths of mice following the inoculation of the 1 100 dilution could legitimately be considered as caused by virus in the organ over and above the amount in the contained blood This excess of virus in an organ might be due to its multiplication or accumulation or to a combination of both these factors The increase was noted in the local lymph glands the left inguinal lymph glands the spleen the liver the adrenal glands and the mesenteric lymph glands Noteworthy was the absence of virus in the kidney and brain

The monkey killed on the 7th day had passed the stage of blood stream invasion consequently the interpretation of the results is more clear-cut All mice dying after inoculation of suspensions of an organ of this animal must be considered as dying of virus present in that organ—either residual virus accumulated there during the time when the blood stream contained virus or virus resulting from multiplication Organs in which virus was demonstrated were the local lymph glands (i e the right inguinal lymph glands) the left inguinal lymph glands the spleen kidney liver adrenal glands retroperitoneal lymph glands and mesenteric lymph glands There was distinct evidence that the amount of virus in the local lymph glands was decidedly less than in the monkey killed on the 5th day of infection The spleen kidney and liver showed only traces of virus whereas the adrenal glands and the mesenteric and retroperitoneal lymph glands showed considerable amounts

The last animal in this experiment namely the one killed 9 days after inoculation like the previous animal in the series had passed the stage of blood stream invasion and showed no demonstrable virus in the circulation at the time it was killed In this animal virus was present in the left inguinal lymph glands the spleen the liver and the mesenteric lymph glands None was demonstrated in the local lymph glands the kidney adrenal gland or retroperitoneal lymph glands The spleen and liver had only traces of virus and the amount present in the left inguinal and mesenteric lymph glands was considerably less than in the previous monkey which had been killed days before

Several points are worth noting in this experiment

There was a large amount of virus in the local lymph glands demonstrable in the animal killed 1 day after inoculation and persisting until the 5th day The most likely explanation for this is either that the virus multiplies in these glands or that it accumulates there because of multi

plication at some other site in the region of lymph drainage H... the experiment gave no evidence of multiplication of the virus in t... at the site of inoculation

2 There was a large amount of virus present in the various lymph g... tested and in the liver spleen and adrenal glands all of which co... either lymphoid or reticuloendothelial tissue

3 The virus persisted in the mesenteric as well as the left inguinal ly... glands after the local lymph glands had become negative

4 Virus was absent from the brain throughout the experiment and fr... the submaxillary and parotid salivary gland and the sciatic nerve on t... two occasions (the 7th and the 9th day after infection) when these orga... were tested To understand the significance of these observations... should be recalled that in monkeys dying of encephalitis due to th... French neurotropic virus these organs are infective

5 Only a small amount of virus was demonstrable at any time in the kidneys

A tentative theory was formulated to account for these observations The virus following intradermal inoculation would be transported by the lymph channels to the local lymph glands where it would multiply From here it would be carried by the lymph vessels to the blood being arrested possibly in other lymph glands before it finally found its way into the blood stream The circulating blood would distribute the virus throughout the body where it would be taken up by other lymph glands the liver and the adrenal glands In the lymph glands and possibly in the liver and the adrenal glands further multiplication would take place

A subsequent experiment but on a larger scale confirmed in the main the previous findings There was also observed the presence of virus in significant amounts in the bone marrow for a considerable period commencing with the time of blood stream invasion and persisting until after the blood had become free of virus

Though these two experiments afforded very little evidence that there was any multiplication of virus in the liver in other experiments definite evidence was obtained that it may multiply in this organ It must be emphasized that the virus content of the liver in all these experiments should be interpreted with caution due to the fact as pointed out previously that an extract of liver tissue has a deleterious action on the virus Nevertheless the conclusion seems justified that the French neurotropic strain does not

multiply in the liver to the same extent as the unmodified French and Asibi strains The highest titer found in the liver of a monkey infected with the French neurotropic virus is 1 20 000 whereas with the Asibi strain titers of 1 10 000 000 have been recorded In the livers of monkeys infected with the French neurotropic strain no pathologic changes were seen either macroscopically or microscopically whereas in monkeys infected with the Asibi virus liver lesions are marked

It is probable that in man vaccinated with the French neurotropic virus alone a method used on a large scale by the French in Africa an infection is produced similar to but milder than that observed in monkeys That essentially the same sequence of events occurs following the method of vaccination introduced by Sawyer Kitchen and Lloyd (1932) has been shown in experiments in monkeys It will be recalled that this method consists in inoculating immune serum before the injection of the French neurotropic virus In monkeys passively immunized prior to the inoculation of virus it was found that the local lymph glands became infective first followed by the iliac and retroperitoneal lymph glands These and the skin at the site of inoculation were the only organs that contained the virus in any amount Other organs in which virus was definitely demonstrated were the spleen on the 3d 5th and 7th days mesenteric lymph glands on the 7th day and the liver on the 5th day Organs which possibly contained traces of virus were the left inguinal lymph glands on the 3d day the adrenal glands on the 7th day and the bone marrow on the 5th and 9th days Mice inoculated with suspensions of these organs as a rule remained well with the exception of the times mentioned in no case did more than one mouse of a group of 12 die

Although no virus was definitely shown to be present in the blood the blood stream must have been invaded early Not only was the serum of the inoculated animals tested for circulating virus but the washed blood cells well That blood stream invasion must have occurred is convincingly own by the presence of virus in the spleen liver and mesenteric lymph nds The apparent absence of virus in the blood may be accounted for in of two ways either virus was present in such small quantities that the hods used were not adequate for demonstrating it or it was masked by presence of antibodies

is evident that monkeys inoculated with immune serum and virus de ed a generalized infection which differed only in degree from that ced by the virus alone It is noteworthy that the quantity of virus in

the organs of passively immunized animals was markedly less than in normal animals The immune serum apparently had no action on the virus *in the skin at the site of inoculation as it could be demonstrated there* throughout the experiment Likewise in the local lymph glands it was present for the first 7 days and possibly even on the 9th day The kidney liver and adrenal gland showed either no virus or only the merest traces The organ that showed the largest amount apart from the skin and local lymph glands was the spleen In this organ virus could be demonstrated on three occasions namely the 3d 5th and 7th days At no time was the amount comparable to that in the local lymph glands The only other organs in which virus was present in any amount were the mesenteric lymph glands on the 7th day *Immune serum apparently has the power of preventing* the full development of the infection The liver is evidently protected by it This observation explains the fact that when monkeys are inoculated with immune serum and the fully virulent Asibi strain they are not only protected from death but they develop an active immunity The immune serum seems to protect the liver from the action of the virus but does not prevent the development of a mild infection which is presumably confined to the lymphoid and possibly the hematopoietic tissues

The leukopenia observed in man and monkeys immunized by the method of Sawyer Kitchen and Lloyd is in all probability due to the infection of the hematopoietic and lymphoid tissues

In the discussion of the pathogenicity for monkeys of the unmodified F W strain of virus the efficacy of the clearing mechanism was pointed out No evidence was obtained to indicate what organs were involved Similar *experiments were undertaken with the French neurotropic virus* In the first of these a normal rhesus monkey was given an intravenous injection of virus Titration in mice indicated that the inoculum contained approximately 350 000 I D Two minutes after inoculation and again after 30 minutes 1 hour 1½ hours 2 hours and 3 hours the animal was bled from the vein and the virus content of the blood was determined by intracerebral inoculation of mice The titer was 1 250 immediately after inoculation After 30 minutes it was 1 17 and then dropped rapidly to 1 7 at 1 hour The titers obtained at 1½ and 2 hours were 1 7 and 1 3 respectively At 3 hours only a trace of virus was recovered The monkey was killed 4 hours after infection and the virus content of the serum and organs was determined Virus was shown to be present only in the serum and spleen and in both only traces were found This experiment confirmed the previous

finding of the efficacy of the clearing mechanism and suggested that the spleen was involved

In an attempt to get more definitive information essentially the same technique was employed using however an inoculum that contained 750 000 000 I D₅₀ The virus content of the serum obtained 2 minutes after inoculation was 1 15 000 The serum titer 4 hours afterward when the animal was bled to death was 1 3 The organs tested and the titer of virus were as follows spleen 1 47 lung 1 43 adrenal gland and bone marrow only a trace the liver kidney mesenteric lymph glands inguinal lymph glands skin and brain contained no virus

The results of this experiment are clear cut The spleen and lung evidently are the organs mainly responsible for the clearing mechanism The adrenal glands and bone marrow may play a secondary role The evidence here is equivocal as only one mouse in each group inoculated with a 10 per cent suspension of these two organs developed yellow fever encephalitis

PATHOGENICITY OF THE 17D VIRUS FOR RHESUS MONKEYS

In the section dealing with the development of the 17D strain of yellow fever virus in tissue culture many particulars will be detailed as to its pathogenicity for rhesus monkeys (p 105) This strain is distinguished from all others by its marked attenuation When it is inoculated intracerebrally into rhesus monkeys it causes an encephalitis which is as a rule nonfatal On subcutaneous inoculation it causes a mild systemic infection which differs from that produced by all other strains of yellow fever in the presence of only minimal amounts of virus in the circulation By simultaneous titration of a 17D virus preparation in mice and monkeys it has been found that the minimal infective dose for monkeys is of the order of one minimal lethal dose for mice This fact alone is adequate to warrant the conclusion that the 17D virus is capable of producing an infection in rhesus monkeys on extraneural inoculation

The course of infection in monkeys inoculated with the French neurotropic the F W and the Asibi virus strains has been described Evidence has been presented to show that the more viscerotropic the strain the higher the concentration of virus in the liver and that all the strains have the ability to infect cells of the lymphatic and hematopoietic systems The organs which seem to play the greatest role in the clearing mechanism are the spleen and lung However some other organs containing reticulo

endothelial cells must also be considered as playing a part in the defense mechanism

In view of the fact that the 17D virus has obviously lost to a considerable extent its virulence for rhesus monkeys in studying the course of infection in these animals the infectivity of the organs was tested in only a 10 per cent dilution Serum was tested undiluted However in order to obtain some idea as to the relative concentration of virus in the different organs from 12 to 24 mice were inoculated with every extract

The plan adopted for determining the mechanism by which the virus spreads throughout the animal body was essentially the same as that used for other virus strains Six monkeys were inoculated intradermally on the ventral aspect of the right thigh with an inoculum containing approximately 550 LD_{50} for mice On the 1st day and every 2d day thereafter until the 11th day a monkey was killed and the infectivity of its organs tested The course of blood stream invasion was determined for all the monkeys

In the first two animals of the series killed on the 1st and 3d days after infection virus had not yet appeared in the blood The next two killed on the 5th and 7th days showed the highest point of blood stream infection The animal killed on the 9th day was in the later stages of blood stream invasion whereas the last monkey of the series killed on the 11th day had passed the stage of blood invasion The organs tested for infectivity were various lymph glands spleen liver kidney adrenal gland bone marrow brain lung and skin at the site of inoculation

No evidence was obtained of any multiplication of virus in the skin Considerably less was present there 3 days after inoculation than after 1 day and from the 5th day onward none was found The highest concentration in the local lymph glands was noted on the 1st day but virus was present there until the 7th day The monkey killed on the 5th day—the 2d day of blood stream invasion—had demonstrable virus in the local lymph nodes the iliac and retroperitoneal glands the spleen the adrenal and the left inguinal lymph glands In none of these tissues was it present in any concentration The monkey killed on the 7th day showed virus in the same organs and in addition in the bone marrow mesenteric lymph glands lung and liver The largest amount was in the bone marrow followed by that in the left inguinal and mesenteric lymph glands The fifth animal of the series killed at the end of the stage of blood stream invasion showed virus in the same organs as the previous animal with the exception that none was present in the liver In this animal the largest amount was in the

mesenteric and left inguinal lymph glands followed by that in the spleen and bone marrow The final monkey of the series had no demonstrable virus in any organ

Throughout the entire experiment there was in no instance a sufficient amount of virus in any tissue extract to kill all the mice inoculated Consequently it seems legitimate to take the proportion of mice becoming infected as an index of the relative amount of virus in a specific organ

On only one occasion on the 7th day was any virus demonstrated in the liver and then only in trace amounts and only one of 22 inoculated mice was stricken with encephalitis The adrenal gland and the lung showed virus on two occasions but both times in extremely small amounts The only organs in which it could be demonstrated in relatively large amounts were lymph glands bone marrow and spleen It was never found in the kidney The conclusion that the virus was actually multiplying in lymphoid tissue and bone marrow is obvious

The pattern of infection of monkeys with the 17D strain of virus is essentially the same as that observed in infections with other strains There is the same sequence of events discernible the multiplication in the local lymph glands the invasion of the blood and localization and multiplication in the other organs However with the 17D strain in contradistinction to all others studied the infection is confined entirely to organs containing lymphoid tissue and bone marrow Furthermore the concentration of virus is far less than that produced by the more virulent strains It is probable that the leukopenia observed (Smith Penna and Paoliello 1938) in persons vaccinated with the 17D virus is the result of infection of the hematopoietic system

SUSCEPTIBILITY OF ADULT MICE

The finding by Theiler (1930b) that white mice are susceptible to intracerebral inoculation of yellow fever virus was soon confirmed by er workers (Sawyer and Lloyd 1931 Dinger 1931) Since then these mals have been used extensively in yellow fever work

n contradistinction to the rhesus monkey the adult mouse with rare ptions becomes infected only when the virus is introduced into the ous system Although all strains of mice are susceptible to an intra ral inoculation of the virus Sawyer and Lloyd (1931) found that the ent strains of mice tested by them varied in degree of susceptibility obtained mortalities ranging from 100 per cent to 50 per cent in

various strains inoculated with a standard amount of virus Suckling mice are susceptible or resistant to an intraperitoneal inoculation of virus, depending on whether they are born to parents belonging to strains which are susceptible or resistant to an intracerebral inoculation Lynch and Hughes (1936), as a result of breeding experiments between two strains of mice, one highly susceptible and the other relatively resistant, came to the conclusion that hereditary factors for resistance to yellow fever encephalitis are present in mice Hybrids of the two strains showed a mortality less than that of the susceptible strain By crossing the hybrid back to the susceptible strain the mortality rate was increased, in backcrosses between the hybrids and the more resistant strain, the rate was lowered As a result of the investigations of Sawyer and Lloyd, the Swiss strain of mice has come into use for yellow fever work

The usual method of producing an infection is by direct introduction *of the virus into the brain However, inoculation into the spinal cord* or into the eye will also produce a typical encephalitis (Theiler, 1930*b*) In the adult mouse, infection very rarely *follows the injection of the virus by* extraneural routes However, if at the time that a large dose of virus is inoculated into the abdominal cavity, the brain is damaged by the injection of *some inert substance, encephalitis will occur (Sawyer and Lloyd 1931)* Following the inoculation of a large quantity of virus into the peritoneal cavity some virus can be demonstrated for a short period of time in the *circulating blood The virus in the blood is in all probability due to absorp*tion from the peritoneal cavity and not to multiplication of virus and release into the circulation By producing a trauma in the brain and consequently *some hemorrhage,* virus which may be circulating has the opportunity of coming into contact with nerve cells and thus initiating the infection, which progresses, producing a fatal encephalitis This observation formed the basis for the development of the intraperitoneal protection test (Sawyer and Lloyd, 1931)

By serial passage of the virus in mouse brains certain predictable events occur If the virus has been highly virulent for monkeys there is a progressive attenuation for these animals Th[illegible]ler, in his original work, tested the pathogenic[illegible] for monkeys of [illegible] virulent French strain after 3, 29, and 42 [illegible] brain pas[illegible] first monkey inoculated with the 3d p[illegible] [illegible]ypical exp[illegible] [illegible]llow fev[illegible] [illegible]e 5th day The second, [illegible] mater[illegible] the [illegible]age, had [illegible] the [illegible] and th[illegible] mal [illegible]eks

afterward this animal was shown to be immune to the original highly virulent strain The third monkey inoculated with the 42d serial passage of the French virus had no fever This attenuation has been confirmed repeatedly Thus Sawyer Kitchen and Lloyd (1932) working with the Asibi strain inoculated monkeys with mouse brain virus representing the 5th 10th 11th 15th 20th and 25th passage A monkey inoculated with the virus of the 5th passage died of yellow fever Of two monkeys inoculated with virus of the 10th passage one died of yellow fever and the other had fever followed by immunity One which received the 11th passage virus died of yellow fever Monkeys inoculated with the 15th 20th and 25th passages had fever and were immunized

The other change induced in the virus by serial passage in the brains of mice is the progressive shortening of the inoculation death period In the earlier passages the shortening is rapid followed by a slower but progressive decrease in later passages This is shown by results obtained with the French virus Mice inoculated with virus of the first five passages died on the 7th and 8th days By the 20th passage most mice died on the 6th or 7th day The time of death in the 75th passage was approximately 5 days Lloyd Penna and Mahaffy (1933) in continuing this series found that the virus eventually became fixed after more than 100 passages in mice when most of the inoculated animals died on the 4th day after inoculation

It has been found that the rate of fixation of yellow fever virus in mice is an inherent characteristic of the particular virus strain A strain such as the French becomes fixed slowly In a duplicate series of serial mouse passages done with the French virus essentially the same results were obtained The Asibi strain on the other hand became fixed very rapidly attaining its maximum virulence for mice after about 25 passages

Neurotropism is an inherent quality of yellow fever virus Bauer has shown that the concentration of Asibi virus in the serum of infected rhesus monkeys is at times such that a billionfold dilution is often capable of producing an infection Titration of the virus content of the serum of infected monkeys by the intracerebral inoculation of mice gives infectivity titers of the same order of magnitude It is quite usual to find that serum from a monkey infected with the Asibi strain can produce a fatal infection when diluted 10 000 000 fold and inoculated in 0 03 cc amounts There is here thus no question of adapting the virus to mouse brain By passage in mice the virulence increases for these animals The difference in the disease picture produced in mice by the unmodified Asibi virus and by

the same virus after numerous mouse brain passages is twofold A mouse inoculated with a given dose of the unmodified virus becomes ill after a relatively long incubation period and the length of illness is usually 2 or 3 days A similar dose of the modified virus produces a very acute illness after a short incubation period It appears as though the major difference between an unmodified virus and virus fixed for mice is that the latter has the ability to multiply at a more rapid rate in the nervous tissue of the mouse From a limited number of observations it would seem that the titer of virus attained in the brain by the time of death is the same whether the infection is produced with an unmodified virus or with one modified by mouse brain passage

The incubation period in a mouse inoculated with a virus is determined not only by the strain but also by the amount of virus in the inoculum Thus the fixed French neurotropic strain when inoculated in large amounts will produce death in about four days whereas mice inoculated with minimal amounts will die on the 7th or 8th day Mice inoculated with the Asibi strain seldom die before the 8th day even when the inoculum contains the virus in high concentration When a small dose of the same virus is given death may occur up to 30 days after inoculation These two strains can thus be readily distinguished by their pathogenicity for mice In titration experiments in mice with fixed strains as well as with most natural strains the average time of death becomes progressively delayed as the virus is diluted With high concentrations all the mice die With dilutions near the end point individual mice begin to escape and on further dilution more mice fail to become infected With strains of virus that behave in this manner the estimation of titer is best obtained by the method of Reed and Muench (1938) This method consists in calculating the theoretical dilution of virus which produces a 50 per cent mortality

With some strains of virus however paradoxical results may be obtained Thus it may happen that of a group of mice inoculated with virus in high concentration very few or even at times none will show signs of infection whereas mice inoculated with dilutions may all die This phenomenon is graphically shown in the following illustration A monkey infected with the highly virulent Jegede strain was bled on the 4th day after inoculation and the undiluted serum as well as five tenfold dilutions were inoculated intracerebrally into groups of mice None of the animals inoculated with the undiluted serum showed any signs of illness and all

remained alive, whereas all of the animals receiving the 1 1 000 dilution became ill and died This phenomenon was repeatedly seen in mice inoculated with the Jegede strain and could be produced at will The unmodified French strain will at times show the same paradoxical phenomenon and it is no doubt due to this irregular behavior that Theiler had some difficulty in adapting this virus to mouse brains This anomalous behavior disappears rapidly on serial mouse brain passage Irregular behavior of this kind has also been observed repeatedly in South American strains Thus it has been noticed on several occasions particularly in attempts to isolate virus from human yellow fever patients by the inoculation of mice that very few if any mice became ill when inoculated with the patient s undiluted serum whereas mice inoculated with a dilution of the same serum became ill Fox (1913) summarized the evidence relating to the irregular behavior of South American viruses He pointed out that workers in Brazil have long recognized that the susceptibility of mice to unmodified virus of jungle origin may not be uniform Mice inoculated with serum from persons suspected of having yellow fever may develop a mild disease from which they recover completely Such animals however as shown by Fox have in all probability had a true infection because it could readily be demonstrated that they had acquired an active immunity Thus immunity was seen in mice that survived the usual period of observation when they were tested by intracerebral inoculation with French neurotropic virus or by examination of their pooled sera in protection tests Of 20 mice surviving after the original inoculation and given a challenge inoculation 17 resisted reinfection and of seven serum pools examined four showed clearly demonstrable protective power Fox found that mice inoculated with either the French neurotropic or the Asibi virus almost invariably died if they showed signs of infection However a few subclinical infections apparently did occur as is indicated by the fact that occasional mice surviving titrations of virus of these two strains resisted reinfection and also that pools of sera from such mice were found to contain protective antibodies Fox concluded that inapparent infections with these two strains were very infrequent

It is clear that titration experiments in mice with strains behaving in such an anomalous manner are difficult to evaluate unless the immunity of all surviving mice is tested In three titrations in mice two with the J Z and one with the O C strain Fox calculated the titers by two methods In the first only the number of mice that died were used to calculate the

LD_{50} by the method of Reed and Muench (1938). *In the second, the mice* that resisted reinfection as well as those that died, were utilized to calculate the minimal infective dose. The LD_{50} of the three titrations were 60, 32 and 28, whereas the minimal infective titers were 2,340, 288, and 1,410 respectively. In these titration experiments there is a suggestion that mice inoculated with the higher concentrations were more likely to have an inapparent but immunizing infection than animals receiving small quantities of virus.

The occurrence of nonfatal infections has also been reported with the 17D strain. Here, however, small doses of virus provoked a significantly higher proportion of nonfatal infections than large doses. It will be recalled that the 17D virus was derived from the Asibi strain by prolonged cultivation in tissue culture. The modification in the pathogenicity of the Asibi virus for experimental animals, induced by prolonged cultivation, is fully discussed in the section dealing with tissue culture.

All evidence indicates that, in the adult mouse, only cells of the nervous system are *susceptible to infection with the virus of yellow fever*. At the time of death, following an intracerebral inoculation, virus can readily be demonstrated in the brain, spinal cord, and sciatic nerve (Theiler, 1930*b*). Blood, liver, spleen, kidney, and testes contain no virus. However, in marked contrast to the organs mentioned is the adrenal gland. This organ contains virus in considerable amounts in mice dying after intracerebral inoculation. It will be recalled that the medulla of the adrenal gland is developmentally associated with the sympathetic ganglia.

That the virus probably travels along the nervous routes was shown by testing the infectivity of various parts of the nervous system at different intervals of time after an intracerebral injection. By the use of the French virus, only partially fixed for mice, it was shown (Theiler, 1930*b*) that the spinal cord is already highly infectious 3 days after an intracerebral inoculation of virus, whereas the sciatic nerve and adrenal gland are noninfectious at this time. Later, on the 5th and 6th days, when the mouse is dying, virus is present all through the nervous system, including the sciatic nerve, and in the adrenal gland.

In addition to the usual mode of producing infection of the nervous system of the mouse, fatal encephalitis can also be produced, according to evidence presented by Findlay and Clarke (1935*a*), if neurotropic virus is instilled into the nares or conjunctival sac. They instilled *into the nares* in approximately 0.03 cc. amounts, a 20 per cent mouse brain suspension

mice thus inoculated 36 showed symptoms of encephalitis in from 2 days Two strains of neurotropic virus derived from the French sibi strains were used The disease picture was similar to that pro by intracerebral inoculation

in attempt to follow the pathway of infection after an intranasal instil of virus Findlay and Clarke tested the infectivity of the blood spleen rious portions of the central nervous system at daily intervals after in a No virus could be demonstrated in the spleen throughout the entire iment In the blood on the 3d day a minimal amount of virus was nstrated On the other days the blood was virus free The cerebral pheres were shown to be infective from the 2d day onward Virus was se shown to be present in the midbrain from the 2d day onward as the hindbrain became infective only on the 3d day There was thus a gradual extension of the virus from the cerebral lobes to the hind of the brain The results of the experiment suggested that the blood n played no role in the distribution of the virus Following the intra instillation of virus the infection presumably traveled up the olfac erves In a search for further evidence as to the possible route Findlay Mahaffy (1936a) found that mice which had been treated with from five en intranasal instillations of picric acid were relatively nonsusceptible instillation of virus as compared with mice not so treated It was known the nasal instillation of various chemicals including picric acid red guinea pigs relatively resistant to an intranasal instillation with e encephalomyelitis virus and rendered monkeys resistant to polio tis virus Findlay and Clarke (1935a) concluded from these findings rom results of experiments with immature mice and monkeys that the ble pathway of the virus from the nasal cavities to the brain was by erineural sheaths As these authors point out however the possibility ect passage of the virus along the axones cannot be excluded

lowing the instillation of virus into the conjunctiva only relatively nimals became infected Thus of 20 mice inoculated in this manner approximately 0 03 cc of a 20 per cent suspension eight developed toms of encephalitis after an incubation period of from 6 to 16 days sting the infectivity of various portions of the brain at daily intervals inoculation Findlay and Clarke obtained confirmatory evidence that erebral lobes are infected before the other parts of the brain Follow he conjunctival route of infection virus was first demonstrated in the

cerebral lobes on the 4th day, whereas the midbrain and hindbrain became infected only on the 5th day

SUSCEPTIBILITY OF BABY MICE

Baby mice in contrast to adults are highly susceptible to yellow fever virus when it is administered by extraneural routes Thus Theiler (1930*b*) found that all mice less than 2 weeks of age died following an intraperitoneal or subcutaneous inoculation of partially fixed French virus The signs and symptoms in baby mice are the same as those seen in adult mice following intracerebral inoculation although the incubation period is several days longer than in adult mice Bugher (1941) studied the influence of age as well as dosage He inoculated baby mice ranging in age from 2 to 13 days subcutaneously with small doses of two strains of yellow fever virus—27 LD_{50} of the French neurotropic virus and 700 LD_{50} of the unmodified Martinez strain With both viruses there was a clear break in susceptibility at the 9th day All but one mouse less than 9 days of age died of yellow fever virus encephalitis In a simultaneous titration of the Martinez strain in baby mice 3 days of age by subcutaneous inoculation and in adult mice by intracerebral inoculation almost identical end points were obtained clearly indicating that baby mice are just as susceptible as adults to this strain of virus The investigations of Bugher were undertaken primarily to determine whether baby mice could be used in mosquito transmission experiments with unmodified strains Previously Davis Lloyd and Frobisher (1932) had used baby mice in transmission experiments with the French neurotropic virus and were able to produce a fatal encephalitis in them by the bite of aegypti mosquitoes infected with this modified strain Bugher exposed 10 two day old mice to the bite of individual aegypti that had fed 20 days previously on a monkey infected with the Martinez strain Seven of the baby mice became ill on the 7th or 8th day after exposure Of the 10 mosquitoes used five were observed to become engorged the others were seen to bite but without engorgement Of the baby mice of the first group three became infected whereas in the second group four of five mice became infected This experiment showed clearly that baby mice could be used to test the ability of mosquitoes to transmit the infection by bite and the method has come into extensive use in transmission experiments

On the hypothesis that the transition from full susceptibility to resistance was gradual Whitman (1943) tested the reaction of mice 14 to 35 days of

age to an intraperitoneal inoculation of the French neurotropic virus It was found that nearly all mice 21 days of age or younger died following the inoculation of 0 02 cc of a 15 per cent suspension of mouse brain infected with this strain This represents a relatively large dose of virus In the older age group tested the number of mice that became infected was less with increasing age The shift in susceptibility with increasing age was rapid as was shown by the fact that it required three times as much virus to kill 21 day old mice as was needed to kill 18 day old mice This observation that mice 18 to 21 days old are intermediate between newborn and adults in their susceptibility to an intraperitoneal injection of yellow fever virus was the basis for the development of an intraperitoneal protection test which had the advantage that no cerebral trauma was required and that small quantities of sera could be used

It has been pointed out that certain virus strains behave irregularly when inoculated intracerebrally into adult mice Bates and Roca Garcia (1945) working with the Colombian Perez strain found that mice 5 days of age were more uniformly susceptible to an intracerebral injection than adult mice The same authors (Bates and Roca Garcia 1946*b*) obtained similar results with the Rodas strain another Colombian virus In a comparative parallel titration in baby and adult mice the end point with baby mice was usually in the next tenfold dilution beyond the final dilution which caused death in adult mice In comparative tests in baby mice 3 days of age inoculated subcutaneously and 5 to 7 day old mice inoculated intracerebrally almost identical results were obtained

While the baby mouse has come to play an important part in yellow fever research and particularly in mosquito transmission experiments very little information is available as to its response to modified yellow fever virus Lennette and Koprowski (1946) found that the 17D strain unlike the Asibi strain from which it was derived was not pathogenic for baby mice on subcutaneous inoculation They made use of this fact to distinguish between these two strains in experiments designed to test possible interference in tissue culture

The distribution of virus in baby mice at death following an extraneural inoculation of virus is the same as that in adult mice after an intracerebral inoculation In such mice virus can be demonstrated in all portions of the central nervous system and the adrenal gland but is absent or present in only minimal amounts in the liver spleen and kidney In an attempt to determine the path of infection in young mice after an intraperitoneal in

jection (Theiler 1930*b*), the infectivity of various organs was tested at intervals after inoculation. The results showed that 24 hours after an intraperitoneal inoculation, the blood was not infective, whereas a pool of liver, spleen and kidney contained some virus, though not any great amount. No attempt was made to determine whether the virus shown to be present in the abdominal organs was on the peritoneal surface of these organs or actually in the tissues. On one of three occasions, virus was present in the brain 24 hours after inoculation.

Forty-eight hours after an intraperitoneal injection, the virus was definitely confined to the nervous system. Liver, spleen, and kidney were entirely free of virus at this time. The results of infectivity tests 3 days after an intraperitoneal inoculation were the same as those of the 48 hour series except that the brain contained the virus in larger amounts.

The infectivity of the sciatic nerve was tested on two occasions: once on the 3d day after injection and the second time on the 6th day, when the mouse was dying as a result of the infection. No virus was definitely shown to be present on the 3d day, whereas on the 6th day it was present in considerable amounts. The difference in infectivity of the sciatic nerve on the 3d and the 6th days, and the absence of virus in the blood at any time, was considered good evidence in favor of the theory of the propagation of the virus along the nerve.

Findlay and Mahaffy (1936*a*) attempted to determine the pathway of infection of the nervous system in baby mice following extraneural routes of inoculation. They tested the infectivity of the blood and different portions of the brain at intervals after an intraperitoneal inoculation of neurotropic yellow fever virus. At 6 and 18 hours after inoculation considerable amounts of virus were present in the circulation, as manifested by the fact that all mice inoculated with blood taken at these hours died. The quantity of virus had diminished by 24 hours and had disappeared by 48 hours. In view of the high infectivity of the blood at 6 and 18 hours, the results of the infectivity tests with the various parts of the nervous system are of doubtful significance. The virus which was demonstrated in the three portions of the brain 6 hours after inoculation was in all probability due to the virus in the blood. However, it is significant that no virus was demonstrated in any portion of the brain 18 hours after inoculation and that at 24 hours only the forebrain, i.e., the cerebral hemispheres, contained virus. At this time the midbrain and hindbrain were virus free. The latter two portions were shown to contain virus 42 hours after inoculation. The conclusion seemed war-

ranted that the forebrain became infected before the other portions As Findlay and Mahaffy had previously shown that adult mice treated by several intranasal instillations of picric acid are rendered relatively nonsusceptible to an inoculation with yellow fever virus by the same route they therefore undertook to determine whether an intranasal instillation of picric acid had any effect on the intraperitoneal inoculation of virus in young mice In five experiments they found that in each case the mortality in the mice treated with picric acid was less than that in the controls 64 per cent of the treated animals survived whereas only 7 5 per cent of the controls lived The results of Findlay and Mahaffy s experiments suggested that in the baby mice inoculated intraperitoneally the virus passes from the blood to the nasal mucosa from which it invades the brain by neural pathways

SUSCEPTIBILITY OF GUINEA PIGS

The finding that rhesus monkeys are susceptible to yellow fever made available a ready source of virus in the laboratory However rhesus monkeys because of their *high cost* are not ideal experimental animals and therefore search was continually made for other susceptible animals The response of the guinea pig was studied by several workers Sellards (1930) reported that he had been able to pass yellow fever virus through three guinea pigs by intraperitoneal inoculation of blood and spleen and concluded that these animals undergo an inapparent infection Sawyer and Frobisher (1932) bled guinea pigs at intervals following an intraperitoneal inoculation of a massive dose of Asibi virus and inoculated rhesus monkeys with the blood They produced infection in the monkeys with blood obtained 16 hours 4 days and 5 days after inoculation but not with blood drawn 8 and 15 days after inoculation They came to the conclusion that in these animals an actual infection occurred and that the recovery of the virus was not due merely to passive storage in their bodies Furthermore by allowing a guinea pig to be bitten by infected mosquitoes these authors obtained evidence that indicated that in all probability active virus was present in the animal s blood 4 days after it had been bitten They also showed that guinea pigs developed antibodies to yellow fever virus While the results obtained by Sellards and by Sawyer and Frobisher indicated that the guinea pig was susceptible to yellow fever by parenteral inoculation it was obvious that this animal was of limited practical value for studies of the disease

After the discovery of the susceptibility of the mouse to yellow fever by

intracerebral inoculation it was soon shown by several workers (Stefanopoulo and Wassermann 1933 Theiler 1933*b* Lloyd Penna and Mahaffy 1933) that guinea pigs are readily infected by the intracerebral inoculation of neurotropic yellow fever virus Most authors have found that the virus can be maintained indefinitely in guinea pigs by brain to brain passage without any shortening of the incubation period However the great regularity in the incubation period and the disease picture seen in mice is not observed in guinea pigs After a variable incubation period of from 4 to 14 days guinea pigs develop a temperature of 40°C or over At this time the animals begin to lose weight and are obviously ill Usually a progressive paralysis develops commencing as a rule in the hind legs The paralysis spreads rapidly and the animal dies after a day or two Recovery has never been observed The irregularity of the disease may be due to variation of susceptibility in these animals The titer of virus in the brain of a moribund guinea pig is always comparatively low and extremely variable The amount of virus in infected brains as tested at regular intervals after injection increases up to the time the animal shows fever The length of the incubation period as well as the duration of fever seems to have no strict correlation with the amount of virus present

The distribution of virus in a guinea pig killed when moribund after an intracerebral injection is essentially similar to the distribution in a mouse killed when sick after an injection with a virus fixed for mice In the guinea pig virus is present at this time in the brain spinal cord and occasionally in the sciatic nerve but not in the blood liver spleen or adrenal gland The spread of virus throughout the nervous system is much slower than in the mouse Thus the spinal cord does not become infective until 5 days after intracerebral inoculation

As far as could be determined the prolonged passage of a neurotropic virus in guinea pig brains did not lead to any change in the virus A virus fixed for mice appeared to be fixed for guinea pigs

The results of early attempts to infect guinea pigs by the intracerebral route with unmodified virus were all inconclusive Theiler (1933*b*) inoculated guinea pigs with the unmodified Asibi strain Most of the inoculated animals showed a rise of temperature and several died after an incubation period of from 5 to 10 days Microscopic study of the brains of those that had died showed evidence of encephalitis which in some cases was quite marked Virus was present in the brain in only small amounts and attempts to maintain it in guinea pigs by brain to brain passage almost invariably failed On

only one occasion could virus be demonstrated in the brain of a second passage guinea pig. However after the Asibi strain had been maintained for 75 brain to brain passages in mice fatal infection could be readily produced in guinea pigs by its inoculation. This fixed Asibi virus was established without difficulty and could be maintained by brain to brain passage in guinea pigs. Attempts to establish the Asibi virus after only one to eight passages in mouse brains were not successful. From these experiments it seemed clear that guinea pigs are only slightly susceptible on intracerebral inoculation to the unmodified or partially modified Asibi strain but are susceptible to this strain fixed for mice.

Although Sawyer and Frobisher and Sellards came to the conclusion that the guinea pig is susceptible to the extraneural inoculation of yellow fever virus, Theiler in his early experiments could find no conclusive evidence of this. Later the results of some additional investigations clearly indicated that there is an enormous difference in susceptibility of various strains of guinea pigs to yellow fever virus. Thus it was shown that several strains are highly susceptible to a subcutaneous inoculation of the unmodified Asibi virus. Animals inoculated with this virus remained well but considerable quantities of virus could be readily demonstrated in the circulating blood. Such animals developed antibodies and, as a rule, were rendered immune to a subsequent intracerebral inoculation of a highly neurotropic strain of virus. Another strain of guinea pig appeared to be entirely nonsusceptible to a subcutaneous inoculation, showing neither virus in the circulation nor the development of antibodies. A still different strain of guinea pig seemed to be intermediate in susceptibility.

By the use of the highly susceptible strain of guinea pig it was shown that following the subcutaneous inoculation of minimal quantities of the Asibi virus a mild systemic infection occurred in which virus was demonstrated in the circulation. In an attempt to follow the course of infection and determine the tissue affinities of the virus, several guinea pigs were inoculated intraperitoneally with 6,000 mouse I.D.$_{0}$ of the unmodified Asibi strain. At daily intervals a guinea pig was killed, and the infectivity of its organs was tested by the intracerebral inoculation of mice. The course of blood stream infection was determined in each animal. The first guinea pig in the series was killed before blood stream invasion. The animals killed on the 2d to the 5th day showed virus in their circulation at the time they were killed, whereas in those killed on the 6th to the 8th day the blood was virus free, although in all three of these animals virus was demonstrated 3 to 5 days

after inoculation The results of the inoculation of mice with the tissue suspensions showed virus to be present in four organs in the liver from the 3d to the 6th day in the mesenteric lymph glands from the 5th to the 8th day and in the spleen and the adrenal glands on the 5th day only No virus was demonstrated in the kidney skeletal muscle bone marrow brain ovary or testis The absence of virus in the brain and its presence in the liver is clear evidence of the viscerotropic affinity of the virus It is of interest that no lesions either macroscopic or microscopic were found in any of the organs Virus persisted in the liver for 1 day and in the mesenteric lymph nodes for 3 days after its disappearance from the blood stream The concentration of the virus in the blood and abdominal viscera was not determined It is apparent however that the viscera did not contain it in any quantity as in no instance did all of 12 mice inoculated with the organ suspensions become infected The type of infection in guinea pigs inoculated with an unmodified virus is thus very much like that produced in monkeys In both liver and lymphoid tissue appear to be involved However whereas in the monkey the bone marrow becomes infected no virus was found in this tissue in the guinea pig Not too much importance should be attached to this observation as all the information available concerning the tissue affinities of yellow fever virus in guinea pigs comes from the one experiment

Studies of the circulating virus in guinea pigs inoculated intraperitoneally with graded doses of the Asibi virus showed that relatively small doses induced infection A more exact method of learning the minimal infective dose was obtained by determining the smallest amount of virus that would stimulate the production of antibodies It was found that with the exception of one animal all guinea pigs inoculated with one or more mouse lethal doses of Asibi virus developed antibodies indicating that this animal is just as susceptible as the rhesus monkey Entirely different results were obtained when the susceptibility of the guinea pig was tested with strains of virus modified by either mouse passage or prolonged growth in tissue culture Thus the French neurotropic virus did not consistently stimulate the production of antibodies unless the inoculum contained more than 10 000 L D 50 for mice The highly viscerotropic Asibi strain is consequently a far more efficient infective agent than the relatively avirulent French neurotropic strain This difference was also observed in studies on the circulating virus following parenteral inoculation of the French neurotropic virus The results of several experiments showed that after the intraperitoneal inoculation of the French neurotropic strain virus was present in the circulating

blood only exceptionally even when the animals were inoculated with relatively large doses Following the intraperitoneal inoculation of large amounts of virus of this strain only the serum obtained 4 hours after inoculation contained virus none was present thereafter This is quite different from the results obtained with the Asibi strain

The pathogenicity of various subcultures of the 17D strain was tested in guinea pigs As it had been found that the determination of the minimal infecting dose as shown by the development of immunity was the most accurate method of ascertaining the pathogenicity of a virus strain this method was employed Guinea pigs inoculated intraperitoneally with the culture virus invariably remained well The minimal immunizing dose of the 38th 64th 95th 127th 212th and 450th subcultures of the 17D strain was determined It will be remembered that the minimal immunizing dose of the Asibi virus the parent strain from which the 17D was derived is approximately 1 I D The number of LD_{50} required to immunize a guinea pig with the various subcultures of 17D virus showed a progression from the earliest subcultures to the later ones Thus only about 1 ID_{50} of the 38th subculture was required to immunize guinea pigs to a subsequent intracerebral inoculation of 1 000 I D $_{50}$ of the French neurotropic virus whereas it required 8 000 LD_{50} of the 212th subculture to render a guinea pig immune No figure was obtained in the experiment with the 450th subculture With this subculture only two of the 36 animals inoculated with from five to half a million ID_{50} for mice developed immunity In two groups of six guinea pigs inoculated with 50 000 and 500 000 ID_{50} respectively one animal in each group developed antibodies and survived the test dose with French neurotropic virus The minimal immunizing dose must consequently be more than 500 000 This latter figure indicates how extreme was the modification produced in the Asibi strain by prolonged cultivation The results would also tend to indicate that this modification as determined by the ability of the virus to infect guinea pigs by parenteral inoculation was progressive Another indication of modification in the Asibi virus by cultivation is the fact that in guinea pigs inoculated with the cultivated virus representing the 95th 127th and 212th subcultures no virus could be demonstrated in the circulating blood

By the use of the susceptible strain of guinea pig it was shown also that these animals are highly susceptible to an intracerebral inoculation of the unmodified Asibi strain and that on cultivation the virus loses its ability to produce a fatal infection when inoculated by this route The minimal

lethal dose for guinea pigs by intracerebral inoculation of the parent Asibi strain was from 1 to 10 LD_{50} for mice The figure rose to 600 LD_{50} for the 38th subculture to fall again to 20 for the 61th subculture and to 3 for the 95th subculture In the succeeding subcultures the 127th 212th and 450th the minimal lethal dose rose steadily The reason for these anomalous results is not known though they may be due to the fact that guinea pigs used for determining the LD_{50} dose of the 61th 95th and 127th subcultures were all young The animals used for the other experiments were adults No data are available concerning the influence of age on the susceptibility of guinea pigs to intracerebral inoculation of yellow fever virus The minimal lethal dose of virus maintained in tissue culture for the 127th 212th and 450th subcultures was 35 500 and more than 10 000 respectively The conclusion seems warranted that the 450th subculture by intracerebral inoculation is far less pathogenic for guinea pigs than either the parent Asibi strain or the various earlier subcultures In the experiment designed to determine the minimum lethal dose of the 450th subculture for guinea pigs by intracerebral inoculation five groups of eight animals each were inoculated with from one to 10 000 mouse LD_{50} None of the animals died The approximate minimum lethal dose must consequently be more than 10 000 mouse LD_{50} However in testing the immunity of these guinea pigs by the intracerebral inoculation of the French neurotropic virus 1 month after the original inoculation it was found that quite a number were resistant The minimum immunizing dose of the 450th subculture by intracerebral inoculation was determined to be approximately 30 It is evident that this immunization must have been due to a nonfatal encephalitis

In a similar manner it was determined that the minimum infective dose for guinea pigs by intracerebral inoculation of the earlier subcultures in all cases was less than 10 LD_{50} for mice It may consequently be concluded that the prolonged cultivation of the Asibi virus produced a significant loss of virulence for guinea pigs but no marked change in its infectiousness by intracerebral inoculation

These experiments have been described here in some detail in order to emphasize the difficulty at times of determining whether or not an animal is susceptible With animals of one species in this case the guinea pig entirely different conclusions would be arrived at depending on the strain of guinea pig the strain of virus and the route of inoculation used By parenteral inoculation a suitable strain of guinea pig is highly susceptible to the Asibi virus Thus on one occasion infection was produced by the bite of a single

infected aegypti mosquito Another strain of guinea pigs would be considered completely resistant to the same virus Similar results are found on intracerebral inoculation Some strains appear to be very resistant to the lethal action of unmodified strains of virus but one strain of guinea pigs was found that developed a fatal encephalitis even when inoculated with a minimum quantity of unmodified Asibi virus

SUSCEPTIBILITY OF HEDGEHOGS

Findlay and Clarke (1931a and b) found that the common European hedgehog *Erinaceus europaeus* is extremely susceptible to yellow fever virus When inoculated with a viscerotropic (Asibi) strain animals of this species invariably died in from 4 to 7 days showing macroscopic and microscopic lesions very similar to those seen in rhesus monkeys infected with yellow fever After seven serial passages in these animals the virus was still virulent for rhesus monkeys

When inoculated with a neurotropic strain hedgehogs became infected and died not only after intracerebral but also after intraperitoneal or subcutaneous inoculation In the livers of such animals focal areas of necrosis were seen similar to but much less marked than the necrosis produced by a viscerotropic strain Serial passage of a neurotropic strain through 10 hedgehogs did not lead to any increased capacity for producing more intense liver lesions

The distribution of virus at death differed somewhat depending on the strain of virus In those animals dead of Asibi infection virus was present in the blood liver spleen kidney and to a less extent in the brain In those dead of neurotropic virus infection the organ showing the greatest concentration of virus was the brain Liver spleen and kidney had less while the blood showed virus only rarely

In these experiments Findlay and Clarke used three different strains of neurotropic yellow fever virus the French after 112 mouse brain passages the Asibi after 118 such passages and the Dakar strain after 226 It is significant that all three strains still had the ability to produce liver lesions in hedgehogs However the cause of death in all probability was encephalitis as the brain was the organ which showed the greatest concentration of virus The histopathologic lesions in the brain however were slight

Findlay and MacCallum (1937a) demonstrated that due to interference neurotropic virus can protect hedgehogs against a viscerotropic strain

Hedgehogs inoculated with mixtures of the two strains died of encephalitis but without extensive liver necrosis or the presence of virus in the blood

At the Yellow Fever Laboratory in New York experience with the hedgehog was limited on account of the difficulty in obtaining this animal The main interest there was to study the course of infection in this unusually susceptible animal For this purpose the French neurotropic virus was chosen and the plan of the experiment was essentially the same as that used for studying the course of infection in rhesus monkeys

Five hedgehogs were inoculated intradermally with approximately 2 000 I D $_0$ The injections were made on the ventral aspect of the right thigh just below the inguinal region On the 1st day following infection and every 2d day thereafter one animal was killed and the infectivity of the serum the right and left inguinal and mesenteric lymph glands the spleen kidney liver adrenal gland heart muscle and brain was tested Except in the case of the serum no effort was made to titrate the virus content of any tissue As a rule 12 mice were given an intracerebral inoculation of the supernatant fluid of a lightly centrifuged 10 per cent suspension of each organ

In the animal killed on the 1st day after inoculation virus could be demonstrated in only the local lymph glands The 2d animal in the series killed 3 days after infection showed a very high virus content in the blood and consequently as no effort had been made to perfuse the animal all the organ suspensions with the exception of the heart muscle contained considerable amounts of virus

The serum of the hedgehog killed 5 days after inoculation contained only minimal amounts of virus as only two of six mice inoculated with the undiluted serum died Significant amounts of virus were demonstrated in all the organs with the exception of the heart and brain but those which showed the most virus as indicated by the fact that all the mice inoculated died were the inguinal glands from both sides the mesenteric lymph glands and the liver Organs which showed a lesser virus content as judged by the fact that not all the inoculated mice died were the adrenal gland the kidney and the spleen

The next hedgehog in the experiment killed on the 7th day showed like the preceding animal minimal amounts of virus in the circulation The organs which contained sufficient virus to kill all injected mice were the inguinal glands from both sides and the spleen The suspensions prepared from the liver mesenteric lymph glands and brain contained enough virus to kill all but one of the inoculated mice whereas the kidney and adrenal

suspensions had a relatively low virus content as is shown by the fact that less than half of the inoculated mice died

The last animal of the series killed on the 9th day was shown by the protection test to have antibodies in its serum at that time Virus was demonstrated in this animal in the brain left inguinal lymph glands spleen mesenteric lymph glands liver and right inguinal lymph glands Organs which were free from virus were the kidney adrenal gland and heart muscle Only in the brain was there sufficient virus to kill all the inoculated mice In the other organs where virus was demonstrated it was present in less concentration than on the 7th day

It is probable that the sequence of events in hedgehogs is similar to that which occurs in rhesus monkeys Hedgehogs inoculated with a neurotropic strain undergo a systemic infection in which the virus probably multiplies in the liver spleen and lymph nodes Unfortunately in this experiment the bone marrow was not studied During the systemic infection the brain becomes involved producing an encephalitis from which the animal dies

On account of the extreme susceptibility of the hedgehog to yellow fever Findlay and Clarke suggested that it is the animal of choice for the isolation of virus This was borne out by the results of a limited series of experiments performed in the Yellow Fever Laboratory in New York where it was found that these animals became fatally infected when inoculated with strains of virus of low pathogenicity for rhesus monkeys However not all the viruses produced a fatal infection That a strain highly pathogenic for rhesus monkeys and hedgehogs can become so attenuated that it no longer produces a fatal infection in either species of animal was shown by Findlay and MacCallum These authors found that by serial passage in mouse carcinoma the unmodified highly virulent French strain became attenuated to the point where it no longer caused the death of rhesus monkeys or hedgehogs Theiler and Smith (1937a) tested the pathogenicity for hedgehogs of two tissue culture variants of the Asibi virus both these variants 17F and 17D had lost to a considerable extent their pathogenicity for rhesus monkeys A hedgehog inoculated with the 81th subculture of the 17F strain died of yellow fever whereas all of six hedgehogs inoculated with the 212th subculture lived and were later shown to have developed antibodies In an experiment in which six hedgehogs were inoculated with the 198th subculture of the 17D culture virus the results were marred by the fact that five of the six animals died of intercurrent infection 12 to 14 days after inoculation Intracerebral inoculation of mice with suspensions of liver and brain from

these hedgehogs failed to reveal the presence of virus In contrast eight hedgehogs inoculated with the parent Asibi strain died in 3 to In all of these, virus was found to be present at death in the liver brain, or in both

SUSCEPTIBILITY OF RABBITS

Several workers have tested the susceptibility of the rabbit to yellow fev Sawyer and Frobisher (1932) showed that these animals responded to inoculation of viscerotropic virus by the development of antibodies, but n virus could be recovered from the blood obtained 2, 4, and 7 days afte inoculation Findlay (1934a) tested the reaction of rabbits to an intracerebral inoculation of neurotropic virus The animals remained well, and the virus disappeared from the brain within 4 days without evidence of multiplication

In a more exhaustive study Whitman (1935) could find no evidence of multiplication of neurotropic virus when it was inoculated either into the brain or intraperitoneally In the blood of rabbits receiving a large infecting dose intraperitoneally, virus was demonstrated, but only in small amounts, 24 hours after inoculation and not thereafter In animals receiving a small infecting dose, no virus was demonstrated at any time These results differ fundamentally from those obtained when susceptible animals are inoculated with this strain of virus Thus, monkeys inoculated intraperitoneally with very large doses will show virus in the circulation for at least 2 days, whereas those inoculated with a small infecting dose will show circulating virus for several days after an incubation period

The results of some additional experiments in the Yellow Fever Labora tory in New York confirmed Whitman's findings An attempt was made to follow the spread of virus in rabbits inoculated intradermally by methods which had proved so successful with other species of animals Rabbits were noculated intradermally on the dorsum of the right hind leg This site of noculation was chosen as it is known that the lymph drainage from this ea goes to the popliteal lymph gland Five rabbits were inoculated At ily intervals one was killed, and suspensions of various organs and the n at the site of inoculation were injected intracerebrally into mice Virus demonstrated in minimal quantities in the skin 24 hours after inocula but not thereafter In the local lymph nodes, it was present only 24 and ours after inoculation There was no evidence of any multiplication in

these organs At no time was any virus present in the blood spleen liver kidney adrenal gland mesenteric lymph nodes or bone marrow

In studying the immune response of rabbits Whitman (1935) showed that these animals developed antibodies very readily The response as determined by antibody titer with rare exceptions was proportional to the amount of virus in the immunizing inoculum Of great significance was the finding that following a rest period of 6 weeks a 2d injection into a previously immunized animal produced a very high titer of serum antibodies

TISSUE CULTURE EXPERIMENTS

FRENCH NEUROTROPIC AND ASIBI STRAINS

The first tissue culture experiments with yellow fever virus were reported by Haagen and Theiler (1932) In preliminary experiments these authors used the method of cultivating minute fragments of tissue on a cover slip over a hollow ground slide Plasma and tissue fragments of various laboratory animals were tested for their suitability The cultures were inoculated with the French neurotropic strain but in spite of good tissue growth the virus disappeared from the majority in a few days The best results were obtained when chick embryo was used as the tissue component Cultivation in series was finally achieved by the use of fluid media in Carrel flasks The tissue component consisted of finely minced chick embryo and the fluid portion was Tyrode s solution containing normal monkey serum In this way two serial cultures were maintained in which subcultures were made every 3 or 4 days the inoculum being 0 5 cc of the fluid portion of the previous subculture The virus maintained its pathogenicity unaltered for mice and one of the serial cultures later reported on by Haagen (1933) was shown to have retained its neurotropic affinity for monkey brain after more than 100 subcultures Two cynomolgos monkeys inoculated intracerebrally with 0 5 cc of the supernatant of the 10th subculture died of encephalitis

Haagen (1934) reported that serum was necessary for the continuous cultivation of the virus and found that adjustment of the medium to a pH between 7 0 and 8 0 was desirable He also found that there was a greater virus content in the tissue fragments than in the supernatant fluid and that an immune serum could prevent the infection of tissue fragments in the culture but did not prevent virus multiplication in cultures already infected

All these results indicated that the French neurotropic virus behaved in tissue culture much like other viruses

As the main object of these cultivation experiments was to determine whether modifications could be induced in the virus by prolonged cultiva tion efforts were directed toward varying the tissue component and espe cially toward cultivating the unmodified strains of virus The first successful cultivation of an unmodified virus was reported by Lloyd Theiler and Ricci (1936) with the highly virulent Asibi strain By using the Maitland type of medium the tissue component of which was minced mouse embryo a suc cessful culture series was initiated Several attempts to cultivate the Asibi virus in the medium that had been found satisfactory for the French neuro tropic virus—one containing minced chick embryo—proved unsuccessful Furthermore even after the establishment of the Asibi virus in a medium containing minced mouse embryo the cultivated virus refused to grow in one containing chick embryo tissue until after 18 subcultures in the mouse embryo medium when a separate branch was initiated containing minced whole chick embryo The series of cultures with the Asibi strain that was established by Lloyd Theiler and Ricci represented the 17th set of tissue culture experiments by these authors Later Theiler and Smith (1937*a*) in order to distinguish the various series of cultures added initials to the ex periment numbers The series cultivated in a medium containing mouse embryo tissue was designated 17E

Lloyd Theiler and Ricci then attempted to start cultures in many diverse kinds of media After the series grown in minced whole chick embryo had been maintained for 58 subcultures two subseries were initiated In one of these the tissue component was modified to the extent that instead of using the whole minced chick embryo the brain and spinal cord were removed be fore mincing and in the other the tissue component consisted of minced chick embryo brain The former strain was designated as 17D and the latter 17D (CEB) A third strain of virus referred to as 17AT was obtained from 17E after 27 subcultures The tissue component of the medium used in this series was minced adult mouse testicle After 70 subcultures in this medium adult guinea pig testicle was substituted for the mouse testicular tissue Of interest at this point is the observation by Lloyd and Mahaffy (1935) and by Smith (1938) that yellow fever can be propagated in mouse testicle in vivo

The numerous culture experiments of Lloyd Theiler and Ricci may be summarized briefly as follows Two strains of yellow fever virus of widely

divergent tissue affinities—the highly virulent viscerotropic Asibi and the highly neurotropic French—were cultivated in media containing either whole minced mouse embryo or chick embryo tissue containing varying amounts of nervous tissue In addition the Asibi strain was cultivated in adult mouse or guinea pig testicular tissue

The results of the cultivation experiments with the French neurotropic strain will be considered first This strain after 305 serial passages in the brains of mice was readily grown for 55 subcultures in a medium containing whole mouse embryo and serum–Tyrode s solution This strain as judged by its pathogenicity upon intracerebral inoculation of adult mice behaved from the beginning to the end of the cultivation period as a fixed neurotropic yellow fever virus the average time of death of mice inoculated with the cultivated virus being about two days later than that of mice inoculated with the parent mouse passage virus

The same strain of French neurotropic virus was cultivated for 110 passages in a medium containing minced whole chick embryo It also behaved consistently from the beginning to the end of the series as a fixed neurotropic virus At each subculture it was injected into the brains of mice and in these mice the time of death was on the average 2 5 days later than in mice inoculated with the parent strain This culture series was run in parallel for 86 passages in which the fluid portion of one series consisted of the usual serum–Tyrode s solution whereas in the other Tyrode s solution alone was used The experiment showed that serum is not essential for the cultivation of yellow fever virus No difference in pathogenicity for adult mice was noted in the two series which differed only in the presence or absence of serum in the fluid portion of the medium

A second strain of the French neurotropic virus which had undergone only 108 passages in mouse brains was readily cultivated for more than 120 subcultures in a medium consisting of minced chick embryo tissue and serum–Tyrode s solution This strain was considered to be only relatively fixed for mice Throughout the experiment mice inoculated intracerebrally with the culture virus died on an average 1 to 1 5 days later than those inoculated with cultures of the more fixed (305 mouse passages) neurotropic strain cultivated in the same medium After 16 subcultures in whole minced chick embryo this strain was propagated in parallel series for 27 passages in the same fluid medium but in different tissue cells—one in chick embryo brain and the other in chick embryo from which the brain and spinal cord had been dissected away The viruses of the parallel series despite the great

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All the monkeys that survived were shown to have developed serum antibodies and were resistant to a test inoculation with the highly virulent parent strain A further indication of attenuation with prolonged cultivation was shown in the progressive loss of ability of the virus to induce fever in the inoculated monkeys In all the monkeys virus was readily demonstrated in the circulating blood

Further evidence that the 17E strain had become attenuated by prolonged culture in vitro was shown by the results of serial passage in rhesus monkeys Commencing with the 92d subculture the virus was passaged in series by intraperitoneal inoculation through 30 rhesus monkeys Of the 30 monkeys inoculated in series six died of typical yellow fever There was a slight but not definite indication that the serial passage enhanced the virulence of the virus for monkeys Of the first 15 animals in the series two died and six showed fever whereas in the second 15 monkeys four died and eight showed fever The significant points of this experiment are the low mortality produced by the cultivated virus and its relative stability on serial passage in monkeys As a routine passage was made with blood or serum obtained on the 3d day after inoculation Throughout the series virus was isolated from the blood stream of surviving monkeys on the 2d 3d and 4th days following inoculation by the intracerebral inoculation of mice From the beginning to the end of the experiment the virus when injected intracerebrally into mice behaved like an unmodified strain that is the incubation period was long and the morbidity period was prolonged The disease picture produced in mice by the 17E virus was thus very like that produced in these animals by the parent Asibi strain indicating that there had been no alteration in the neurotropic affinity

The experiments reported by Lloyd Theiler and Ricci on the pathogenicity of the 17E strain of culture virus were made with the first 130 subcultures Theiler and Smith (1937*a*) continuing this work reported on the results of the inoculation of rhesus monkeys with material from the 179th to the 206th subcultures Of 10 monkeys inoculated subcutaneously with culture virus one died of extraneous causes while nine remained well and were all shown to have become immune to the parent Asibi strain Of significance in this experiment was the fact that in all the inoculated monkeys virus was demonstrated in the blood in considerable amounts for relatively prolonged periods of time In seven of the monkeys virus was shown to be present for as long as 6 days indicating that even after prolonged cultivation was still able to produce a relatively severe systemic infection The ex

differences in the amount of nervous tissue provided for growth showed no variation in their pathogenicity for mice when inoculated intracerebrally.

The results of all these experiments with neurotropic strains of yellow fever virus tended to show that the virus is extremely stable, as no indication was obtained that any modification occurred when it was cultivated for long periods of time in media containing either mouse embryo or chick embryo tissues. The fact that mice inoculated with the culture virus died on an average several days later than those inoculated with the parent strain is probably due to the relatively low titer of virus in the cultures.

Turning attention next to the results obtained in the experiments with the Asibi strain, it was soon observed that the cultivated virus was becoming modified. As this virus was at first grown in a medium containing minced mouse embryo tissue (17E), most of the early work was done with the virus grown in this medium. As pointed out above, the affinities of yellow fever virus may be roughly divided into neurotropic and viscerotropic. The neurotropic affinity is most readily shown by the intracerebral inoculation of mice, while the viscerotropic affinity is shown by the extent to which the virus can multiply when inoculated extraneurally into a rhesus monkey. What is perhaps the best index of the viscerotropic affinity of the virus is the titer it attains in the circulating blood of rhesus monkeys. A highly viscerotropic strain like the Asibi produces a fatal infection. In fact, during the 6½ years between its isolation from Asibi and its cultivation in mouse embryo tissue, the virus was transferred, with intermittent periods of life in mosquitoes and preservation in the dried state, through 53 passages in rhesus monkeys. Irrespective of whether transmission was effected by mosquitoes or by syringe inoculation of blood or serum, all of 59 rhesus monkeys so inoculated died of yellow fever. Five animals in the series, which would in all probability have died, were killed. The complete history of the virus from the time of isolation in West Africa until its establishment in tissue culture is given in the article by Lloyd, Theiler, and Ricci (1936).

The pathogenicity of the 17E culture virus for rhesus monkeys was tested at irregular intervals. The fact was soon obvious that this strain was becoming altered to such an extent that on parenteral inoculation it failed to cause fatal yellow fever in these monkeys. Thus, of 12 rhesus monkeys inoculated intraperitoneally with virus from the 5th to the 25th subculture, seven died of yellow fever; conversely, of 10 monkeys inoculated with virus from the 26th to the 49th subculture, only one died, and of 16 monkeys inoculated with cultures from the 58th to the 109th passage, all remained well.

All the monkeys that survived were shown to have developed serum anti bodies and were resistant to a test inoculation with the highly virulent parent strain A further indication of attenuation with prolonged culti vation was shown in the progressive loss of ability of the virus to induce fever in the inoculated monkeys In all the monkeys virus was readily dem onstrated in the circulating blood

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dence considered so far indicated that the Asibi virus grown in mouse embryo tissue had become attenuated for monkeys when inoculated by extraneural routes and also that its neurotropism for mice had not altered

Experiments to determine the neurotropic affinity of this virus for monkeys were reported by Lloyd Theiler and Ricci (1936) and Theiler and Smith (1937*a*) Ten monkeys inoculated intracerebrally with material from the 35th to the 203d subculture all died of encephalitis Two of these animals inoculated respectively with material from the 35th and 82d subcultures showed typical lesions of yellow fever in their livers The consistent death produced in monkeys by the intracerebral inoculation of the 17F virus does not necessarily indicate that the cultured virus had become more neurotropic The parent Asibi strain when inoculated intracerebrally into rhesus monkeys produces death from visceral lesions However if the animals are inoculated intracerebrally with the virus and at the same time receive an intraperitoneal injection of immune serum they die of encephalitis It would seem therefore that the unmodified Asibi strain possesses both viscerotropic and neurotropic affinities for monkeys

As an intraspinal inoculation is perhaps a less severe test for neurotropism Lloyd Theiler and Ricci investigated the pathogenicity for monkeys of the 17E culture virus administered by this route Of 10 monkeys receiving material from the 41st to the 76th subculture all had fever and three died Two of these inoculated with the 45th subculture died with visceral lesions whereas one inoculated with the 76th subculture died of encephalitis These results were in marked contrast with the uniformly fatal outcome in monkeys inoculated intraspinally with the French neurotropic virus (Lloyd and Penna 1933*a*)

In this place it may be pointed out that these culture experiments were undertaken in the hope of finding a strain of yellow fever virus suitable for human vaccination less virulent than the French neurotropic strain then in use As the results of the prolonged culture of the Asibi virus in mouse embryo tissue clearly showed that this cultured virus was definitely less neurotropic than the French neurotropic virus currently employed for human vaccination it was decided to use the cultured Asibi strain for human immunization However as the experiments in monkeys indicated that this culture virus though markedly attenuated for these animals continued to produce a fairly severe systemic infection it was considered still too pathogenic to be used without the *simultaneous inoculation* of immune serum Consequently the method of vaccination introduced by Sawyer

Kitchen and Lloyd was changed only in that the 17F strain was substituted for the French neurotropic one

VIRUS 17AT GROWN IN TESTICULAR TISSUE

The 17AT strain of virus which was grown in a culture medium containing minced adult mouse testicle was derived from the 17F culture at the 27th subculture During the early period of cultivation no evidence was obtained that any modification for mice had occurred The disease in mice resembled in all respects that produced by the parent Asibi strain Furthermore the rate of fixation in mice by serial passage was the same as that produced by the original strain An exact comparison was possible as the original source virus was available in the dried state Two serial passages in mice were undertaken one with the 42d subculture in testicular tissue and the other with the source Asibi virus using the same strain of mice for 23 passages

A second serial passage in mice was initiated with the virus grown in testicular tissue for 160 subcultures At the same time a control serial passage was carried out with the unmodified Asibi strain Analysis of these two serial passages showed that the cultivated strain was significantly less neurotropic than the parent Asibi strain as manifested both by the average incubation period and by the rate of fixation It was apparent therefore that the prolonged cultivation in testicular tissue had produced a loss of virulence for the nervous tissue of mice The conclusion was drawn at the time that this loss of neurotropism was due in all probability to the prolonged cultivation of the virus in the absence of nervous tissue (Theiler and Smith 1937*a*)

The pathogenicity of this strain for rhesus monkeys was tested at irregular intervals On intracerebral or intraspinal inoculation it invariably produced death from encephalitis Lloyd Theiler and Ricci (1936) tested the pathogenicity of the 30th and 35th subcultures by intraspinal inoculation whereas Theiler and Smith (1937*a*) used the 160th subculture The results of extraneural inoculation indicated that the degree of attenuation was more marked than that observed in the 17F strain None of 10 monkeys inoculated with material from the 30th to 13d subcultures in testicular tissue died of visceral yellow fever Furthermore in some of the animals only minimal amounts of virus could be demonstrated in the circulating blood

On the limited amount of evidence available the changes induced in the Asibi virus by prolonged cultivation in testicular tissue in vitro may be sum

marized as follows there was a marked loss of viscerotropism for monkeys, without at the same time any obvious change in neurotropism for these animals there was a slow loss of neurotropism for mice

VIRUS 17D GROWN IN CHICK EMBRYO TISSUE

The Asibi strain of virus grown in mouse embryo tissue for 18 subcultures was used to initiate a series of cultures in which the tissue component was minced whole chick embryo The strain grown in this medium is known as 17D (WC) After further cultivation through 58 subcultures in whole chick embryo two new series were started In one, the tissue component consisted of minced chick embryo brain only, and the strain grown therein was called 17D (CEB) In the other the tissue component was minced chick embryo from which the brain and spinal cord were removed before mincing, the resulting strain was designated as 17D At the time these two culture series were started, the series in which the tissue component consisted of minced whole chick was temporarily discontinued After a very marked attenuation had been observed in the 17D strain, the series in minced whole chick was started again from desiccated virus representing the 64th subculture from the beginning of the culture series and the 47th subculture in whole chick tissue A study of these three parallel cultures was undertaken to determine whether the very marked changes that had been noted in the 17D strain were due to the absence of nervous tissue in that series All three sets consisted of chick embryo, but differed in the amounts of nervous tissue In the following discussion of this study the number of cultures is in each case reckoned from the beginning of the culture experiments with the Asibi virus in mouse embryo tissue, unless otherwise stated

To verify the presence of virus at each subculture mice were inoculated intracerebrally with the supernatant fluid used for making a transfer to fresh media The impression was obtained that the mice inoculated with the 17D virus showed a longer incubation period and a longer period of sickness than mice inoculated with the 17E strain, which was being used in human vaccination As the incubation period in mice depends not only on the strain of virus employed but also on its concentration, the results of numerous titrations were analyzed These showed quite clearly that the 17D strain was less neurotropic for mice than the other culture viruses under investigation

To confirm this conclusion in another way, the rate of fixation when the virus was passed serially in mice was determined Virus in the 176th

subculture of the 17D strain was used to initiate the series As a control similar series were undertaken with the 17E strain after 157 subcultures with the 17AT after 160 subcultures as well as with the unmodified Asibi virus The average time of death at each passage with the different viruses was calculated All four mouse passages showed a progressive shortening of the time from inoculation to death However the rate of fixation varied considerably It was highest with the parent Asibi strain and lowest with the 17D strain There was no marked difference between the parent strain and the 17F strain The rate of fixation of the 17AT strain was intermediate between that of the parent strain and the 17D strain indicating that it had become less neurotropic for mice but not to the same extent as 17D

In order to get some indication as to the time when the loss of neurotropism for mice appeared in the 17D strain serial passages in mice were begun with the 114th subculture At this time the virus had been maintained for 96 subcultures in chick tissue and during the last 38 subcultures only minimal amounts of nervous tissue were present in the medium The rate of fixation was greater and the average time of death significantly shorter in mice inoculated in series with the virus from the 114th subculture than in those inoculated in series commencing with the 176th subculture The loss of neurotropism for mice was evidently progressing in the 17D series Both strains eventually became fixed for mice

Inoculation of monkeys by neural routes showed that the 17D virus lost its power of producing fatal encephalitis between the 89th and the 114th subculture (Theiler and Smith 1937*a*) Thus six monkeys inoculated intraspinally with virus from the 89th subculture died of encephalitis in from 10 to 13 days In all considerable amounts of virus were demonstrated in the circulation In contradistinction four monkeys inoculated intracerebrally with the 114th subculture lived Numerous monkeys have since been inoculated intracerebrally and the above mentioned finding has been confirmed However more extensive experiments have shown that though as a rule monkeys inoculated intracerebrally with the 17D virus will develop a nonfatal encephalitis an occasional monkey dies The incidence of fatal infections is from 5 to 10 per cent and varies as will be pointed out later in different parallel cultures of the 17D virus

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strated Such monkeys were shown to develop specific antibodies and to be solidly immune to the highly virulent Asibi strain

After extensive experiments in monkeys the 17D strain was used for human vaccination It was considered that the loss of both viscerotropic as well as neurotropic affinities as demonstrated in monkeys made this the virus of choice for human vaccination This conclusion has in the main been borne out

In the parallel series of cultures in which minced whole chick tissue (17D WC) and chick embryo brain (17D CEB) were used no such marked attenuation occurred Both these viruses after several hundred subcultures produced fatal encephalitis in monkeys and both produced a rather severe visceral infection as judged by the amount of virus in the circulating blood These results suggested that the amount of nervous tissue was the conditioning factor which produced the change as loss of neurotropism occurred only in a medium containing minimal amounts of nervous tissue It was felt that this finding was so important that confirmation was desirable

Consequently three new series of tissue cultures were initiated by Theiler Virus taken from the series maintained in chick brain only (17D CFB) and from that in whole chick embryo (17D WC) was grown in series in cultures the tissue component of which contained minimal amounts of nervous tissue and conversely the 17D virus which had been grown in a medium containing minimal amounts of nervous tissue was transferred to a medium containing chick embryo brain only Whereas in the original three sets of cultures planned to determine the possible influence of nervous tissue on the cultivated virus the 76th subculture of the Asibi strain in tissue culture was used this second series was begun with the 212th subculture At the time of the conclusion of the experiment more than 200 subcultures in the new series had been made At intervals monkeys were inoculated intracerebrally to determine the neurotropism of the cultivated virus The results showed that no modification had occurred Thus the 17D strain cultivated for more than 200 subcultures in chick embryo brain only had not become more neurotropic and the virus at the beginning and the end of the series was essentially the same in pathogenicity In like manner the other two strains which it will be recalled produced fatal encephalitis in monkeys at the beginning of these culture experiments did not lose this neurotropism even though maintained for more than 200 subcultures in a medium containing minimal amounts of nervous tissue The conclu

sion was obvious that the relative amounts of nervous tissue present in the media had not produced any demonstrable change

The reason for the relatively rapid change noticed in the 17D strain which occurred between the 89th and 114th subcultures was and still is completely unknown However these experiments indicate that once the mutant had occurred it was relatively stable Further evidence of the stability of the 17D virus and the role of nervous tissue in modifying the neurotropic affinities of this virus was given in three serial passages in nervous tissue Two of these were in mouse brains and the other was in the developing chick embryo in which the virus is known to multiply to a large extent in the brain The two mouse brain passages were begun with the 114th and the 176th subcultures which represented the 38th and 100th subcultures respectively in a medium containing minimal amounts of nervous tissue For the serial chick embryo passage the 173d subculture was used The behavior in mice of the serial passage has already been recorded Material from all three serial passages was tested at irregular intervals by inoculation into the brains of monkeys All of nine monkeys inoculated with virus from the 18th to the 120th embryo passages remained well Similar results have been reported by Fox and Laemmert (1947) who showed that prolonged embryo-to-embryo passage of 17D virus failed to produce any significant alterations in virus character

The pathogenicity for monkeys by intracerebral inoculation of virus of the serial passage in mice initiated with the 114th subculture was tested on three occasions Monkeys inoculated with the 61st and 106th mouse brain passage virus remained well while two animals inoculated with the 168th passage virus died of encephalitis in 11 days In the blood of both the latter animals a considerable amount of circulating virus was demonstrated for several days

The pathogenicity of the series started with the 176th subculture was tested by intracerebral inoculation in monkeys after 20 40 106 133 and 195 mouse brain passages The animals receiving material from the 20th and 40th passages remained well The monkey inoculated with the 133d passage virus died from extraneous causes on the 20th day Circulating virus was present for several days At death no virus could be demonstrated in the brain or liver The one monkey inoculated with virus of the 106th passage and two inoculated with that of the 195th passage died of encephalitis in 8 to 11 days At death virus was isolated from the brain of all three but not from the liver In the blood of both the animals inoculated with

virus of the 195th mouse brain passage a considerable amount of virus was demonstrated for the first 3 days after inoculation

These experiments indicate that the 17D strain of virus can be converted into a neurotropic one by prolonged serial mouse brain passage By serial mouse brain passage the virus became fixed for these animals and acquired the capacity of producing a fatal encephalitis in monkeys Of particular significance is the finding that by serial passage in mice the two 17D strains have not only become more neurotropic but also apparently more viscerotropic The amount of circulating virus in the monkeys inoculated with the 168th and 195th mouse brain passages of the two series was more than is usually present in monkeys inoculated with the 17D virus The evidence though highly suggestive, is not conclusive, as no efforts were made to titrate the virus in the circulation In all four monkeys inoculated with the late mouse passage 17D strain virus was demonstrated in the blood on from 3 to 4 successive days immediately following inoculation In all of them sufficient virus was present for 2 or 3 successive days to kill all the inoculated mice On the other hand in monkeys inoculated intracerebrally with the 17D strain virus did not appear in the blood until several days after inoculation and was seldom present in sufficient concentration to kill all the inoculated mice

The results of these serial mouse brain passages of the 17D virus are in contrast to the changes induced in the parent Asibi virus when it is propagated by this method The Asibi virus when maintained in mouse brain passages rapidly becomes highly neurotropic for monkeys acquiring the capacity to kill these animals with fatal encephalitis after approximately 35 passages

The results of the tissue culture experiments with the Asibi virus may be summarized as follows In one of the series of cultures a sudden modification occurred the evidence is that this change was not due to the relative absence of nervous tissue in the medium nor could chick embryo tissue per se be responsible as the change occurred in only one of six series of cultures containing chick embryo tissue

Whatever the reason for the marked change produced in the 17D virus the desired attenuation had occurred A virus which was less virulent than the two then in use for human vaccination had been produced Extensive experiments with monkeys showed that these animals could be rendered solidly immune to a subsequent inoculation of virulent virus even when the immunizing dose was minimal in amount Monkeys inoculated subcu

taneously with the 17D strain showed no signs or symptoms of infection That they had however undergone a true infection could be readily shown by the presence of minimal amounts of virus in the circulation and by the development of antibodies

This culture strain was consequently introduced for human vaccination without the simultaneous inoculation of immune serum In a preliminary study Theiler and Smith (1937*b*) showed that reactions in man were either absent or minimal and that satisfactory antibody response was obtained Smith Penna and Paoliello (1938) made a more thorough and more extensive study of the reaction in man The results were eminently satisfactory and the 17D virus has come into large scale use To date several million persons have been vaccinated

While the results have in the main been good two episodes have occurred which indicate that the 17D virus can become further modified In the first of these the observation was made that the vaccine failed to produce an immunity in some of the persons vaccinated i e a further loss of viscerotropic affinity had occurred In the second it was observed that clinical encephalitis was produced in a small percentage of the vaccinated persons i e an apparent increase of neurotropism had taken place (Fox Lennette et al 1942) The first of these episodes occurred at a time when it was thought that the 17D virus was stable and no standardized method of vaccine production had been introduced Vaccines in the early days were usually made by the inoculation of the developing chick embryo with the supernatant fluid of the current subculture of the virus The result was that the virus in the vaccines differed widely in the length of time it had been cultivated The failure to produce immunity in a considerable proportion of vaccinated persons in Brazil occurred following the use of vaccines made in Rio de Janeiro from the 17D virus which had been in tissue culture for more than 300 subcultures (Soper and Smith 1938*b*) Previous satisfactory vaccines had been made with virus which had not been in cultures for as long a time It was consequently thought that on prolonged cultivation the virus had become progressively attenuated so that it had eventually failed to produce an infection in man That this was not necessarily so was shown by Smith Calderon Cuervo and Leyva (1941) who compared the immune response of groups of individuals inoculated with vaccines prepared in the Yellow Fever Laboratory in New York from the 17D strain after 212 and 450 subcultures respectively No difference was observed between the two groups The conclusion was obvious that on prolonged cultivation of the

17D virus in Rio de Janeiro a further attenuation had occurred whereas similar cultivation in New York had not led to such a change It thus became evident that in two parallel series of similar cultures minor but definite changes might occur In an attempt to throw some light on the loss of antigenicity observed in the Rio strain of 17D virus Bugher and Smith (1944) cultivated the virus for 57 subcultures in the presence of immune serum No evidence that this procedure produced any change in the virus was obtained

As a consequence of the occurrence of clinical cases of encephalitis in several individuals following the use of certain batches of vaccine Fox and Penna (1943) made an exhaustive study of the pathogenicity for monkeys of the various derivative substrains of the 17D virus used in Brazil Vaccines are as a rule submitted to a safety test before being used for human inoculation This consists in inoculating rhesus monkeys intracerebrally with the vaccine and determining the duration and amount of virus in the blood Any increase of neurotropism would be shown by the severity of the encephalitis produced whereas an increase in viscerotropism manifests itself by an increase in the amount of virus in the circulation An analysis of the response of 177 rhesus monkeys inoculated as routine vaccine controls showed that in spite of their common derivation the various substrains of the 17D virus had developed slight but definite differences Substrains were shown to differ in their neurotropic as well as their viscerotropic affinity The latter affinity as noted above was measured by the amount and duration of virus in the blood of inoculated monkeys There was a correlation between the degree of viremia and the immune response The greater the blood stream invasion the greater the immune response There was a certain correlation between the findings in monkeys and observations in man Thus monkeys inoculated with two substrains designated as 17D ? and 17DD *high* not only had very little circulating virus but also showed very low serum antibody response The immune response in man inoculated with vaccines prepared from the same two substrains had been found unsatisfactory The 17D substrain which had been found to produce several encephalitic reactions in man was shown to cause an unusually high incidence of encephalitis in monkeys

The variation in pathogenicity of different substrains of the 17D virus and the consequent variation of the response in man inoculated with these substrains led to the standardization of the manufacture of vaccine At

present a large seed lot of virus is prepared thoroughly tested ir
and stored in the refrigerator. Successive batches of vaccine are :
the seed lot

FURTHER EXPERIMENTS WITH UNMODIFIED STR

Culture experiments with various unmodified strains of yellow
were undertaken at a time when the Asibi strain had been cul
some time and had shown loss of viscerotropic virulence when
minced embryo mouse tissue (17F) The extreme attenuation p
the Asibi strain grown in chick tissue (17D) had not yet been
The 17F virus was at the time in use for human vaccination, bu
still considered too pathogenic to be used alone immune seru
ministered simultaneously The change induced in the Asibi
prolonged cultivation was confined to a loss of viscerotropic a
neurotropic affinity remained unaltered a fact which was cor
great importance Moreover, an adequate attenuation of the vi
affinity had not occurred Smith and Theiler (1937) reasoned
unmodified virus possessing less marked viscerotropic and neuroti
ties could be maintained in tissue culture the requisite amount
tion might occur Various strains were on hand which, on anim
tion, were shown to be less neurotropic and viscerotropic than
The technique which had been so successfully applied to the cul
this strain was repeatedly tried with four other unmodified strain
nine unsuccessful attempts to adapt the F W strain were made Ii
experiments could virus be demonstrated in the primary cultu
instance, virus was present in the second subculture At least
attempts were made with three other strains None of these e
gave promise of success

Numerous experiments in which tissues from monkeys were
ended in failure The most promising results were obtained by
bone marrow and adrenal gland However, in no instance was
demonstrable beyond the first subculture

The successful cultivation of the unmodified Asibi virus in
containing mouse embryo tissue suggested to Smith and Thei
the mouse embryo there must be some group of cells which :
favorable medium for the propagation of the virus On the o
the fact that the concentration of the virus in mouse embryo tis

was never very high seemed to indicate that the proportion of such cells was relatively small in the whole embryo With a view to determining the organ for which the virus had the greatest predilection embryos were infected in utero with the Asibi virus and the relative virus content of the various organs was noted The method used was similar to that described by Woolpert (1936) in his study of bacterial infections in mammalian embryos Four days after inoculation the embryos were removed their tissues were pooled and the virus content was determined by the intracerebral inoculation of adult mice Tissues thus tested were brain liver and other viscera skin placenta legs and tails The results showed that while there was a wide variation in the distribution of the virus throughout the body the brain contained a higher concentration than any other organ as no end point had been reached in a dilution of 10^{-5}

Attempts were accordingly made to cultivate unmodified strains of virus in a medium in which the tissue component consisted of minced mouse embryo brain These proved highly successful Seven different strains of yellow fever virus were tried They ranged in pathogenicity from the highly virulent French strain to viruses low in neurotropic and viscerotropic properties which were newly isolated from persons with jungle yellow fever

It was soon observed that the viruses maintained in the mouse embryo brain appeared to become more neurotropic for adult mice and the possibility of cultivating them in other tissues was investigated At first minced whole mouse embryo was tried In experiments with four different strains this substitution was unsuccessful when efforts were made to transfer virus from the 5th and 6th subcultures in mouse embryo brain to whole mouse embryo When the virus strains had grown from 20 to 25 subcultures in mouse embryo brain however they were adapted to the whole mouse embryo with greater facility

Meanwhile the marked attenuation of the Asibi strain had been found to occur in a medium whose tissue component was minced chick embryo containing minimal amounts of nervous tissue (17D) Consequently attempts were made to cultivate the new strains in a similar medium These were at first unsuccessful However after longer periods of cultivation in mouse embryo tissue it became possible to maintain the viruses in media whose tissue components were prepared from chick embryos from which the brain and spinal cord had been cut away before mincing

Only two of these series of cultures were continued for a long time—the French viscerotropic and the J S S Three different parallel culture series

were maintained consisting respectively of mouse embryo brain minced whole chick embryo and minced chick embryo containing minimal amounts of nervous tissue

At irregular intervals the pathogenicity of the cultured virus was tested by the intracerebral inoculation of rhesus monkeys (Smith and Theiler 1937) The results that were produced with the highly pathogenic French virus showed that its viscerotropism was rapidly lost A monkey inoculated with the 6th subculture in mouse embryo brain died of typical visceral yellow fever The monkey inoculated with the 12th subculture of this series remained well whereas the one inoculated with the 23d subculture died with signs of encephalitis Autopsy revealed typical yellow fever lesions in the liver as well Of eight monkeys inoculated with virus from the 43d to the 187th subculture seven died of encephalitis and one remained well All the monkeys inoculated with virus grown in chick tissue irrespective of the presence or absence of nervous tissue died of encephalitis Five animals were inoculated with the virus grown in minced whole chick embryo from the 60th to the 146th subculture and six with virus grown in minced chick embryo containing minimal amounts of nervous tissue representing the 60th to the 147th subculture The results confirmed those obtained with the 17F virus in that a marked attenuation in the viscerotropic affinity had occurred but there was no such extreme attenuation as was observed in the 17D strain On the contrary it appeared that an actual increase of neurotropism had taken place in all three parallel cultures irrespective of the quantity of nervous tissue in the medium

The J S S strain of virus was maintained for over 300 subcultures in the three different series Forty six per cent of the monkeys inoculated with material up to the 340th subculture died of encephalitis The highest incidence (15 of 20 monkeys) of fatal encephalitis was produced by the virus grown in whole minced chick embryo containing minimal amounts of nervous tissue Next in order was the virus grown in mouse embryo brain which caused death from encephalitis in seven of 17 inoculated monkeys The virus grown in minced whole chick embryo proved to be the least neurotropic as it produced fatal encephalitis in only five of 22 monkeys inoculated Taking the results of the three culture series together there was a suggestion that with prolonged cultivation the neurotropism for monkeys tended to decrease Thus of 15 monkeys inoculated with material from the first 100 subcultures 10 died of encephalitis whereas of 24 animals inocu

lated with virus from the 200th to the 340th subculture only nine died While these figures are suggestive they are not conclusive

The viscerotropism of the J S S virus cultivated in media containing minced whole chick and minced chick tissue containing minimal amounts of nervous tissue was tested by inoculation of monkeys subcutaneously with graded doses of the culture virus Of four monkeys inoculated subcutaneously with material from the 252d subculture of the virus cultivated in chick tissue with minimal amounts of nervous tissue three had fever and all survived All developed serum antibodies The amount of virus injected into the monkeys in terms of LD$_{50}$ mouse intracerebral doses varied from 200 000 to 200

Similar experiments conducted with the 276th subculture of the J S S virus grown in whole minced chick tissue gave somewhat irregular results Of four monkeys inoculated subcutaneously with decreasing amounts of virus ranging from 1 000 to 4 LD$_{50}$ two developed serum antibodies These two were the monkeys that received the largest and the smallest infective doses respectively On repetition with the same virus preparation four animals were given a subcutaneous inoculation with doses of virus ranging from 133 000 to 133 LD$_{50}$ All the animals remained well and none developed antibodies That these monkeys received active virus was shown by the fact that four other monkeys inoculated intracerebrally at the same time with the identical virus preparation all developed antibodies The four monkeys that failed to develop antibodies after the subcutaneous inoculation were given a second inoculation of the same virus preparation in equivalent doses Only in the two that received the two larger doses was there any evidence of the presence of antibodies and then only in small amounts

The results of these experiments leave no doubt that the J S S strain had with prolonged culture in a medium containing whole chick embryo become so attenuated in its viscerotropic affinity that it at times completely failed to produce an infection even when administered subcutaneously in relatively large doses The same virus however is still able to produce an infection sometimes even fatal when inoculated into the brain This modification is the most extreme that has so far been produced in any yellow fever virus The loss of infectivity of the J S S strain for monkeys inoculated subcutaneously recalls a somewhat similar though not so marked change that occurred in one of the substrains of the 17D culture virus which it will be recalled became so attenuated for man that it failed in a fair proportion of cases to produce immunity

INFECTION OF THE DEVELOPING CHICK EMBRYO

first reports of the successful infection of the developing chick em ith yellow fever virus were made by Elmendorf and Smith (1937) authors utilized for their preliminary experiments a strain of virus had been cultivated for a prolonged time in a medium containing l chick embryo tissue They found no difficulty in maintaining this y serial passage in chick embryos using as the inoculum the embryo the membranes or a suspension of embryonic tissue As a routine : was made by direct inoculation of the embryo itself The source ised in these preliminary experiments was the 16₃th subculture in embryo tissue of the 17D strain of culture virus After 18 serial pas n the developing chick embryo its pathogenicity was tested by the rebral inoculation of four rhesus monkeys One of these animals l signs of encephalitis and the other three merely had fever after an tion period of 10 days All survived and were later found to have ped antibodies and to be immune to a test dose of virulent yellow irus This was the first observation that a marked attenuation had ed in the 17D strain of virus To determine whether this attenuation e to passage in chick embryo or whether it had already taken place tissue culture series monkeys were inoculated intracerebrally with ture material which had been used as a source for initiating the serial) passages It was found that the marked attenuation was present in lture virus The inoculation of other monkeys with rehydrated ma rom earlier subcultures demonstrated that the attenuation was pres the 114th subculture

ow fever strains modified either by mouse brain passage or by tissue : can be readily propagated in the developing chick embryo How inmodified strains are not so easily adapted Elmendorf and Smith after several failures succeeded in cultivating the unmodified Asibi ench strains The inoculation of monkeys with material representing assages showed that no modification of virulence had occurred Penna oussatche (1939) on the other hand made the significant observation he cultivation of the Asibi virus in the developing chick led to a d attenuation similar to that which had been observed in the 17D These authors were unable to establish the Asibi virus directly in

the chick embryo but after 19 passages in a culture medium containing minced mouse embryo infection was obtained and could be maintained in series without any difficulty The pathogenicity of the virus propagated in the developing chick was tested by the intracerebral inoculation of rhesus monkeys with material from the 19th 29th 39th 47th 49th and 63d chick embryo passages The monkey inoculated with the 19th passage died of visceral yellow fever The animal inoculated with the 29th passage showed signs of encephalitis but recovered and lived Monkeys inoculated with the later passages all lived Here apparently was a change such as occurred in the 17D strain of virus

In further studies undertaken to ascertain *whether this phenomenon* could be reproduced at will three additional series of passages were made In one of these a similar loss of neurotropism occurred Although the loss of viscerotropic virulence was constantly observed in the course of all four experiments the ability to induce fatal encephalitis in rhesus monkeys was lost only twice

Fox and Laemmert (1947) made an exhaustive study of yellow fever virus infection in the developing chick As the chick embryo had come into extensive use for the large scale manufacture of yellow fever vaccine these authors confined their attention mainly to infections with the 17D strain of virus Only the more significant of their findings will be discussed here They tested the susceptibility of the chick embryo by various routes of inoculation The greatest susceptibility was observed when the virus was inoculated onto the chorioallantoic membrane or by a periembryonic stab Inoculation into the yolk sac or allantoic cavity was not nearly as efficacious In testing the susceptibility of embryos to infection with different strains of virus it was shown that unmodified strains are able to infect embryos only when large doses are inoculated whereas modified strains are infectious when inoculated in very small doses In following the course of infection in eggs inoculated on the chorioallantoic membrane these authors noted an early multiplication of the virus at the site of inoculation followed by an invasion of the blood stream and infection of the embryo Multiplication appeared to occur in all the tissues of the embryo with the highest concentration in the brain and muscles The maximum titer was observed on the 3d or 4th day but virus was found throughout the embryonal period though in decreasing concentrations and was present in the blood of about half of the chicks at the time of hatching The mortality and hatching rate of infected embryos was related to their age at the time of

inoculation There was a high mortality among embryos inoculated when less than 11 days of age Deaths occurred from four to seven days after in oculation, and almost none of the embryos hatched However embryos older than 13 days at the time of infection hatched nearly as well as un infected controls, though the time of hatching was delayed by a few days In the hatched chicks, antibodies developed only in those in which virus was present in the blood at the time of hatching

Fox and Laemmert (1947) studied the effect of prolonged embryo passage upon the 17D and French neurotropic strains and found that no significant alterations in virus character occurred

SUSCEPTIBILITY OF THE HATCHED CHICK

Very little information is available concerning the susceptibility of the hatched chick to the virus of yellow fever Sawyer and Frobisher (1932) showed that adult fowls reacted to the inoculation of a large dose of Asibi virus by the development of antibodies

Linhares (1943) tested the susceptibility of young chicks to the French neurotropic virus by various routes of inoculation They were found to be susceptible by intracerebral, intraperitoneal, and intradermal routes, but showed no symptoms of infection However the presence of virus in the circulating blood and the development of antibodies following inoculation with small doses of virus afforded conclusive evidence that infection and multiplication of virus had occurred The susceptibility was highest in newly hatched chicks and decreased rapidly with age The largest amount of virus in the circulation was found in chicks inoculated when 1 day old In birds 10 days of age virus was seldom found in the blood, and then only in those that had received a large dose

In chicks inoculated by the intracerebral route, virus could be demon strated in the brain for at least 15 days Following an intraperitoneal inocu lation the experimental findings suggested that an initial multiplication of virus occurs at some extraneural site resulting in circulating virus It then becomes localized in the brain where it persists until the 10th day Organs in which virus was demonstrated before its appearance in the brain were the lung liver, spleen, and kidney

Laemmert and Moussatché (1943) tried to infect chicks with the Asibi strain of virus Two attempts to establish the unmodified virus were un successful However, a strain which had been maintained in tissue culture

for 19 subcultures and 22 passages in the developing chick embryo was readily established in chick brains This virus at the time it was established in chicks was still pathogenic for rhesus monkeys The series was maintained in chicks for 102 passages by brain to brain inoculation Throughout the series no passage animal showed any signs of illness Virus persisted in the inoculated chick brain up to 10 days and in some instances up to 13 and 14 days after inoculation but appeared to be in greatest concentration on the 5th and 6th days In the majority of infected chicks virus could be demonstrated in the blood for periods of time ranging from 1 to several days In no instance was it demonstrated after the 6th day Following the inoculation of virus by the subcutaneous route no circulating virus was demonstrated nor was the antibody response as regular as after infection by the intracerebral route However it is significant that an antibody response was observed in some chicks inoculated subcutaneously with minimal amounts of virus suggesting that infection must have occurred

During the course of the passages the virus lost its power to kill monkeys through visceral lesions but was not altered appreciably in its pathogenicity for mice Mice inoculated intracerebrally with passage virus throughout the series had encephalitis after an incubation period of from 5 to 7 days *Monkeys inoculated intracerebrally with material from the 27th* 41st and 98th passages had fatal encephalitis This virus in fact behaved in rhesus monkeys like a mouse brain adapted virus which has a considerably shorter incubation period in mice

GROWTH OF THE VIRUS IN TUMORS

Findlay and MacCallum (1937*d*) found that yellow fever virus has a marked affinity for the cells of mouse tumors Preliminary experiments were made with two readily transplantable tumors of mice one a sarcoma and the other a carcinoma The virus was able to persist in the carcinoma for 12 to 14 days after inoculation *but not in the sarcoma In attempts to determine* whether the virus could persist continuously in symbiosis with the tumor cells transplantation of the infected tumor was made at intervals of 10 to 12 days Although in one instance a neurotropic strain was carried for five passages this method was abandoned because the growth of the virus produced necrosis of the tumor cells which thus failed to grow when transplanted into fresh mice The serial passage of virus in tumor tissue was eventually obtained in the following manner Actively growing tumors were

inoculated After 5 or 6 days the infected tumors were removed and from them a 10 per cent extract in serum saline was made and used to inoculate other actively growing tumors In this manner three virus strains were maintained in series The three strains were the French after 100 mouse brain passages the 17D strain of tissue culture virus and the unmodified French strain No evidence of any change was noted in the pathogenicity of the first two strains after 50 and 60 passages respectively However a very marked loss of pathogenicity for rhesus monkeys and hedgehogs was induced in the unmodified French virus This strain is highly pathogenic for both these species of animals The progressive loss of pathogenicity is indicated by the results of inoculation of monkeys by the subcutaneous route Two of three monkeys inoculated subcutaneously with virus of the 10th intra tumor passage died of yellow fever Only one of three monkeys inoculated with virus from the 20th tumor passage died All of six monkeys inoculated with virus representing the 40th and the 60th passages survived

The attenuation of the virus for hedgehogs appeared to be somewhat less rapid than for the monkeys All the hedgehogs inoculated subcutaneously died of yellow fever except the three inoculated with the 60th passage virus and one of three injected with the 40th passage virus Findlay and MacCallum pointed out that the changes induced in the unmodified French strain were essentially the same as had been reported to occur in the unmodified Asibi strain after prolonged cultivation in tissue culture (17E) that is there was a marked loss of viscerotropism but no change in the neurotropic affinity as determined by intracerebral inoculation into mice Of interest is the observation that yellow fever virus had a marked affinity for neoplastic cells and localization occurred even if the virus was inoculated subcutaneously or intraperitoneally

PROPAGATION IN MOUSE TESTICLE

Lloyd and Mahaffy (1933) were the first to attempt to propagate yellow fever virus in mouse testicle Working with the French neurotropic strain *they were able to maintain the virus through nine consecutive passages but* had considerable difficulty in continuing the passages on account of bacterial contamination

Lloyd Theiler and Ricci (1936) were unsuccessful in passing the unmodified Asibi virus in series in mouse testicles However they had no difficulty with the same strain after cultivation in vitro The virus used to

initiate the series had been cultivated for 27 subcultures in a medium consisting of mouse embryonic tissue and for 37 subcultures in mouse testicular tissue This virus was transferred at weekly intervals for more than 20 passages

Smith (1938) succeeded in bringing about the propagation of four unmodified strains of yellow fever virus by serial passage in mouse testicles The virus was present in the highest concentration about 7 days after inoculation and thereafter it gradually diminished and disappeared entirely by about the twenty first day The mice showed no signs of infection but on microscopic examination of the testicles slight local lesions were discernible In titration experiments it was shown that mouse brain and mouse testicle are almost equally susceptible to yellow fever virus Immunity to a subsequent intracerebral inoculation following an intratesticular inoculation was somewhat irregular

The main object of Smith s work was to determine whether the prolonged growth of yellow fever virus in mouse testicle led to any modification of the virus In a series of passages of the unmodified French strain the pathogenicity for monkeys was tested at intervals Of nine monkeys inoculated by various routes with virus from the 10th to the 40th passage seven died of visceral yellow fever Subsequent to the publication of Smith s work the serial intratesticular passage of the French strain was continued The pathogenicity of the 69th passage was tested in two monkeys one by intracerebral and the other by intraperitoneal inoculation Both died on the 6th day with typical lesions of visceral yellow fever

The Asibi strain was also successfully established by Smith in serial passage in mouse testicle Virus representing the 11th passage inoculated subcutaneously into a monkey produced death from yellow fever in 5 days The series was maintained in mouse testicles for 136 passages On four occasions the pathogenicity for monkeys was tested A monkey inoculated subcutaneously with virus of the 35th testicular passage remained well Of two monkeys inoculated with the 58th passage virus one that was inoculated into the brain lived whereas the other inoculated intraperitoneally died of yellow fever Two monkeys inoculated intraperitoneally with virus from the 64th passage died of visceral yellow fever One of two monkeys inoculated intracerebrally with virus of the 134th passage died on the 10th day At autopsy the liver of this animal appeared normal but on microscopic examination an occasional Councilman body was seen Lesions of encephalitis were present in brain sections Two monkeys inoculated sub-

cutaneously with the 136th testicular passage virus lived Both sl
febrile reactions of several days duration and considerable amounts
culating virus the highest titers being 1 10 000 and 1 250 in the tw
mals respectively The evidence scanty though it is indicates that the
strain on propagation in mouse testicle did become somewhat atter
for rhesus monkeys though at a very slow rate It is however sign
that the rate of attenuation of the Asibi virus propagated in vivo in
testicle is far less than that observed when the same strain is passed in
brains

THE INTERFERENCE PHENOMENON

Hoskins (1935) made the significant observation that when rhesus
keys were inoculated parenterally and simultaneously with two stra
yellow fever virus one highly viscerotropic and the other neurotropic
usually lived The strains of virus used were the unmodified Asibi ar
French neurotropic The protective action of the neurotropic virus
be readily demonstrated not only when it was given simultaneously
the Asibi virus but also when it was administered up to 24 hours afte
virus Thus of 15 animals inoculated with the two strains simultane
13 survived Of 21 animals in which the interval between inoculation
two strains was from 16 to 24 hours 12 were protected When the
tropic strain was given 18 hours after the viscerotropic there was n
tection This latter observation is by no means surprising in view
short incubation period of the Asibi virus in rhesus monkeys inoc
with a large dose of this virus The viscerotropic strain was usually
lated subcutaneously and the neurotropic intraperitoneally Howeve
route of inoculation appeared to make little difference Hoskins ga
explanation for this interference phenomenon though it was show
no antibody was present in the infected mouse brains used as a sou
neurotropic virus

Findlay and MacCallum (1937*a*) confirmed and extended the findi
Hoskins that monkeys inoculated subcutaneously or intraperitoneally
a mixture of neurotropic and viscerotropic strains survived though c
monkeys inoculated with the viscerotropic virus alone died of yellow
These authors also presented evidence that monkeys inoculated int
brally with mixtures of the two viruses died with lesions of encep
and not with necrosis of the liver From these results they conclude
while the neurotropic strain can interfere with the viscerotropic strain

inoculated parenterally no interference occurs when the mixtures are inoculated directly into the nervous system. Similar results were obtained when mixtures of neurotropic and viscerotropic strains were injected intracerebrally into mice. In mice inoculated with mixtures containing a uniformly high concentration of viscerotropic virus and varying dilutions of neurotropic virus the average time of death was the same as in mice inoculated with dilutions of the neurotropic virus alone. From these results Findlay and MacCallum concluded that in the mouse brain the viscerotropic strain did not interfere with the neurotropic strain.

Findlay and MacCallum (1937*a*) likewise presented evidence that a neurotropic virus could protect hedgehogs against a viscerotropic one. The European hedgehog became infected and died following the parenteral inoculation of either strain. The viscerotropic strain produced an acute fatal infection with marked liver necrosis. At death virus was present in the blood. The animals inoculated with the neurotropic virus died of encephalitis. At death there was a slight amount of liver necrosis; virus as a rule could not be demonstrated in the blood but was present in the brain. Of eight hedgehogs inoculated subcutaneously with mixtures of the two strains seven died with encephalitis but without extensive liver necrosis and one lived. Death occurred in from 7 to 16 days after inoculation and no virus could be demonstrated in the blood. The three control hedgehogs inoculated with the viscerotropic strain alone died in 5 or 6 days with extensive liver necrosis and hemorrhage into the stomach; virus was present in the blood of all of them.

Experiments at the Yellow Fever Laboratory in New York confirmed the work of Hoskins and of Findlay and MacCallum. Monkeys inoculated intraperitoneally with mixtures composed of varying amounts of both virus strains lived provided that the inoculum contained neurotropic virus in a *hundredfold greater concentration than the viscerotropic virus.* By the use of frozen and desiccated preparations of the two strains of known titer the required concentrations could be made at will. *With the letters N and V denoting respectively the two virus strains,* the French neurotropic and the viscerotropic Asibi, the following mixtures in terms of L D for mice were found to show the interference phenomenon: 100 000N + 1 000V; 10 000N + 100V; 1 000N + 10V. If the proportion of the Asibi virus was increased no interference took place and the animals died of visceral yellow fever. But all mixtures, irrespective of the proportion of the two strains, when inoculated intracerebrally invariably produced death after acute illness. In

fact it was repeatedly found that when mixtures were inoculated intr cerebrally death occurred after a shorter period of time than when the san mixtures were inoculated intraperitoneally

The lack of interference between the two strains when inoculated intra cerebrally into mice was confirmed The two strains used in these experi ments can be readily distinguished in mice Depending on the concentration of virus the French neurotropic strain kills mice in from 4 to 8 days whereas the Asibi even in the highest concentration seldom produces death in 8 days Mixtures of virus always killed mice in the time expected de pending on the concentration of the neurotropic virus In titrating known mixtures of the two strains the average time of death invariably corre sponded to the estimated concentration of the neurotropic virus unless the viscerotropic strain was in excess In these cases the average time of death of mice inoculated with the dilutions showed a sharp break at the end point of the neurotropic virus The average time of death of the mice beyond this point was prolonged corresponding to the concentration of the Asibi strain in these dilutions By diluting mixtures of the two viruses and inoculating mice the viscerotropic strain could be readily recovered apparently pure if it was in excess in the mixture As the two strains ap parently do not interfere with each other in the mouse brain it follows that in mice inoculated with a mixture although they die in a period cor responding to the concentration of the neurotropic virus the viscerotropic strain must multiply during the time from inoculation to death That this is so is shown in the experiment reported below

Three mixtures containing the two strains in varying proportions were passaged in mice and monkeys All three of these mixtures contained a uniform concentration of the neurotropic virus—1 000 LD per 0 03 cc the viscerotropic concentrations were 10 10 000 and 10 000 000 LD_{50} per 0 03 cc The first of these mixtures (1 000N + 10V) was inoculated by the intracerebral route into mice and by the intraperitoneal route into a mon key The monkey remained well as was to be expected since the proportion of the two strains in the inoculum was such as to allow interference to occur Serum obtained from this animal on the 3d day was inoculated into two other monkeys one by the intraperitoneal and the other by the intracere bral route Both of these animals died of yellow fever with typical liver esions The results indicate that by one passage in a monkey of a mixture f the two viruses their relative ratios had become altered to the extent at the proportion of viscerotropic strain had increased a clear indica

tion that this strain multiplies more rapidly in the monkey than the neurotropic strain The mice inoculated intracerebrally with the original mixture became ill after an incubation period characteristic of the neurotropic strain The virus in their brains was used to inoculate a second group of mice and two rhesus monkeys one of which was injected intracerebrally and the other intraperitoneally As was to be expected the latter animal survived indicating that the relative proportion of the two viruses was still such that interference was possible The monkey inoculated intracerebrally died with lesions of visceral yellow fever proof that one mouse brain passage had not eliminated the viscerotropic strain Virus from this monkey was passaged by intraperitoneal inoculation through three more monkeys All three of these died presenting lesions typical of visceral yellow fever The virus isolated from the blood of the third monkey of this series was titrated in mice The mice inoculated with undiluted serum all died on the 5th and 6th days indicating that neurotropic virus was still present while those inoculated with the higher dilutions died after an incubation period consistent with the viscerotropic strain Two monkeys were inoculated with the 2d passage in mice of the original mixture (1 000N + 10V)—one into the brain and the other into the abdominal cavity As was expected the animal inoculated intraperitoneally lived whereas the one inoculated intracerebrally died on the 7th day Pathologic examination showed lesions of encephalitis but only minimal or questionable lesions in the liver

The second mixture to be studied contained 1 000 LD_{50} neurotropic and 10 000 LD_{50} viscerotropic virus (1 000N + 10 000V) With the viscerotropic strain in excess as was to be expected the rhesus monkey inoculated intraperitoneally died of visceral yellow fever This mixture was also submitted to two mouse brain passages and virus from each passage was inoculated into two monkeys one by the intracerebral and the other by the intraperitoneal route The two monkeys inoculated extraneurally lived indicating that even after one mouse brain passage of a mixture containing viscerotropic virus in excess this ratio is reversed a clear indication that the neurotropic virus multiplies in mouse brains at a greater rate than the viscerotropic The death with liver lesions of both monkeys inoculated intracerebrally indicates that even after two mouse brain passages some viscerotropic virus was still present

The third mixture containing the viruses in the proportion of 1 000N to 10 000 000V per 0 03 cc was tested in the same manner as the first two mixtures A rhesus monkey inoculated intraperitoneally with this third

mixture died of visceral yellow fever Five serial mouse brain passages w
also made with the mixture and two monkeys were inoculated one in
the brain and the other intraperitoneally with virus from the 1st 2d ar
5th passages The behavior of the virus in mice during the five passage
was typical of a neurotropic one All three monkeys inoculated into the
brain with the mouse brain passage virus died after an acute illness from
the 4th to the 6th day following inoculation In all typical lesions of vis
ceral yellow fever were present indicating that even after five mouse brain
passages some viscerotropic virus was still present Of the three monkeys
inoculated intraperitoneally only one the animal receiving the 1st passage
virus died of visceral yellow fever In this instance evidently one mouse
brain passage of the mixture containing viscerotropic virus in great excess
(1 000N + 10 000 000V) had not altered the relative proportions enough so
that interference could take place It required an additional mouse passage
for the neurotropic strain to become sufficiently in excess so that when
inoculated intraperitoneally into a monkey interference could occur and
protect the animal

It is known that when a viscerotropic strain is passaged in mice it loses
its viscerotropism Therefore in the serial passage in mouse brains of a
mixture containing both strains it is of importance to know at what rate
viscerotropism is lost due to the natural attenuation of the viscerotropic
virus Consequently serial passage in mice of this strain alone was carried
out and its pathogenicity was tested periodically in monkeys All of six
monkeys inoculated intracerebrally with virus from the 2d to the 12th
mouse brain passages died of typical visceral yellow fever A monkey inocu
lated intraperitoneally with the 10th mouse brain passage likewise died of
visceral yellow fever In summation it may be said that if monkeys are
inoculated peripherally with a mixture of the two strains the viscerotropic
virus multiplies at a faster rate than the neurotropic whereas the reverse
is true if the mixture is inoculated into the brains of mice

In attempting to unravel the sequence of events in monkeys inoculated
with mixtures of the two strains the virus concentration in the blood was
determined at daily intervals following inoculation It was found that the
concentration of both types could be determined provided the viscerotropic
strain was in excess In animals inoculated intraperitoneally with mixtures
in the requisite proportions so that interference took place and the animals
survived the titers of both strains were always low with the viscerotropic
invariably in excess The highest titer observed was 1 000 In animals in

which no interference took place, and which consequently died of visceral yellow fever, very high titers of the viscerotropic strain were obtained. The concentration of neurotropic virus in the serum of these animals was approximately the same as in animals inoculated parenterally with this strain alone.

An entirely different picture was observed in monkeys inoculated intracerebrally with the mixtures. After a brief illness all these animals died and many showed lesions of visceral yellow fever. In their blood the neurotropic virus attained, as a rule, an unusually high concentration. Thus as an example an animal inoculated intracerebrally with a mixture containing an estimated 100,000 LD_{50} of the neurotropic strain and only 10 LD_{50} of the viscerotropic died in 6 days. In its blood the titer of the neurotropic strain was found to be 1,000,000 and that of the viscerotropic slightly higher. These high concentrations of neurotropic virus were also at times found when the neurotropic strain was inoculated intracerebrally and the viscerotropic intraperitoneally. Though the number of observations is limited, this extreme multiplication of the neurotropic strain was not observed when the routes of inoculation were reversed, that is, when the neurotropic virus was inoculated intraperitoneally and the viscerotropic intracerebrally. In fact, in the latter case, there is some evidence that interference can take place. Thus an animal inoculated with 100,000 LD_{50} of neurotropic virus intraperitoneally and 1,000 LD_{50} of viscerotropic virus intracerebrally died of encephalitis on the 10th day. At no time did the virus in its blood reach a titer above 1 000.

The death of monkeys following an intracerebral inoculation of mixtures of virus, after a short incubation period, looks like the opposite of interference. An indication of this phenomenon is seen in the results obtained by Findlay and MacCallum when they inoculated four monkeys intracerebrally with mixtures of virus. Two control monkeys inoculated with the neurotropic virus alone died of encephalitis in 12 and 14 days respectively. Two other control monkeys inoculated by the same route with the viscerotropic virus died of typical yellow fever in 5 and 6 days. The four animals that received the mixtures of virus died in 7, 7, 7, and 8 days of encephalitis, with no evidence of visceral lesions.

In the New York laboratory series the average time of death of monkeys inoculated intracerebrally with mixtures of virus was 4½ days, the extremes were 3 days and 7 days after inoculation. It will be recalled that death resulted even with mixtures that do not lead to the death of the animals

when inoculated intraperitoneally because of the interference that occurs in the latter case

The unusually high titer of the neurotropic strain in the serum of ani mals inoculated intracerebrally with mixtures of virus is never seen in animals inoculated with the neurotropic strain alone No explanation has been found for this

In the interpretation of the interference phenomenon two facts seem pertinent The first is that the two strains must be inoculated in the requi site proportions the neurotropic virus must be greatly in excess of the viscerotropic The second fact is that both viruses multiply in the liver cells However the neurotropic strain though able to multiply in these cells produces no lesions whereas the viscerotropic strain produces necrosis The interference phenomenon probably consists of the prevention of the invasion of a sufficient number of liver cells to insure protection of the animal against hepatic necrosis and death It seems likely that the strain of virus which reaches the cell first prevents invasion of that cell by the other one In this particular instance a liver cell invaded by a neurotropic virus particle is protected against invasion by a viscerotropic virus particle If different liver cells are invaded each with one variety of virus which multi plies to be released after a time there is a continuous competition between the two strains If the neurotropic strain can maintain its lead and protect enough hepatic cells until the immune mechanism comes into play so that the animal lives interference is said to have taken place

In support of this hypothesis is the fact that no interference between the 17D and Asibi strains of virus takes place in the rhesus monkey even when the 17D strain is inoculated in great excess It will be recalled here that in monkeys infected with the 17D strain the virus is present in the liver in minimal amounts only indicating that it has lost to a great extent the power of infecting and multiplying in hepatic cells

All strains of yellow fever virus are immunologically identical However it seems improbable that the interference of one strain with another as observed in monkeys is due to immunologic processes Conclusive evidence on this point was obtained by Findlay and MacCallum (1937*a*) who were able to demonstrate that a similar interference took place between the viruses of yellow fever and Rift Valley fever These viruses according to all available evidence are not related immunologically Yet the authors ound that seven out of 11 monkeys survived the inoculation of viscerotropic ellow fever virus when it was given 2 hours after an intraperitoneal inocu

lation of Rift Valley fever virus The virus of Rift Valley fever produ monkeys only a short nonfatal disease associated with a few scattered of necrosis in the liver The results of this experiment left no doubt the virus of Rift Valley fever could protect monkeys against yellow f and that its pathogenic action in the monkey is very similar to that of yel fever virus

In the mouse, however, the two viruses differ markedly. Adult mice oculated by extraneural routes with the unmodified virus of Rift Valle fever are extremely susceptible and develop a fatal infection with marke necrosis of the liver It will be recalled that yellow fever virus when in oculated peripherally into adult mice does not cause any infection, and the animals remain well Findlay and MacCallum (1937a) showed that mice could be protected to some extent from the virus of Rift Valley fever by the inoculation of yellow fever virus These authors tested the protective action of the French neurotropic virus by intraperitoneal inoculation preceded or followed by an inoculum of Rift Valley fever virus They found that if the yellow fever virus was inoculated before or at the same time as the Rift Valley fever virus, the mice were partly protected The degree of protection, however, was usually slight, causing only a delay in the death of the animals In a small percentage, complete protection was obtained However, when the order of inoculation was reversed the protective action was not seen if Rift Valley fever virus was given 1 day before the yellow fever virus nor did inoculation of mice with normal mouse brain, or mouse brain containing inactive yellow fever virus give any protection against Rift Valley fever virus These results would indicate that the protective action was due to active yellow fever virus and suggests that this virus has some affinity for the liver cells of the mouse but is unable to multiply in this organ Rift Valley fever virus, on the other hand, not only has an affinity for liver cells but is able to multiply in these cells to the extent of producing death in mice from liver necrosis These observations suggest that the union of yellow fever virus with "receptors" on the cell surface prevents the infection of the cell by the Rift Valley fever virus and thus protects the animal

In investigations performed in the Yellow Fever Laboratory in New ork, a similar interference phenomenon between the viruses of dengue nd yellow fever was observed in rhesus monkeys When monkeys are in ulated intraperitoneally with dengue virus from human sources they are otected against subsequent subcutaneous inoculation of viscerotropic yel fever virus, if the interval between the two inoculations is 1 or 2 days

This protection is shown by the survival of the monkeys. If the interval between the inoculations is more than 3 days the animals are not protected and die of visceral yellow fever. Monkeys immune to dengue are not immune to yellow fever, though such animals as a rule die later than control animals following inoculation with viscerotropic yellow fever virus. This suggests that there is a slight immunologic overlap between these two viruses, and protection tests in mice have given confirming evidence that such is the case. A yellow fever immune serum has a slight but definite protective action in mice against a mouse-adapted dengue virus. The reverse is also true: a dengue immune serum has some protective action in mice against yellow fever virus. The degree of immunologic overlap, however, is not adequate to explain the marked protective action of dengue virus in monkeys. This protective action must be due to interference.

By the use of tissue culture methods Lennette and Koprowski (1946) showed interference between the 17D strain and various other strains of virus. They demonstrated that cultures infected with the 17D strain not only inhibited the growth of the Asibi strain but also that of the immunologically distinct West Nile, Venezuelan equine encephalomyelitis, and influenza A viruses. While these viruses show a great diversity of tissue affinities in adult animals, this may not necessarily be the case in embryonic cells such as those of mouse and chick embryo used by Lennette and Koprowski in their cultures. The mechanism of interference here may be of the same nature as postulated above.

SPONTANEOUS VARIATION IN YELLOW FEVER VIRUS

The discussion so far has been concerned with variation induced in yellow fever virus by certain procedures, such as animal passage or growth in tissue culture. Some of these procedures are invariably followed by predictable changes. Thus, maintenance of a yellow fever virus by serial mouse brain passage induces an enhanced neurotropism for mouse brain and a loss of viscerotropic affinity for rhesus monkeys. Prolonged growth in tissue culture likewise induces a loss of viscerotropic affinity. Other changes have occurred in the virus, however, which it has not been possible to repeat at will. For example, in spite of numerous attempts the marked attenuation observed in the Asibi virus, referred to as the 17D strain, has never been duplicated. But what appeared to be a similar change occurred in the Asibi

virus by passage in the chick embryo This modification was noted twice in four separate serial chick embryo passages (Penna and Moussatche 1939) Bauer observed a change of this kind in the A S strain of yellow fever virus which had been highly pathogenic for monkeys and suddenly lost this characteristic It will be noticed that these apparent sudden changes all entail a loss of virulence

The reverse phenomenon the acquisition of viscerotropism by a neurotropic virus has been reported by several observers Thus Stefanopoulo (1932) recorded that when two monkeys were inoculated intracerebrally with the 161st mouse brain passage of the French virus one died of yellow fever encephalitis as was to be expected but the other died with visceral lesions A suspension of liver tissue from the latter animal was inoculated by the subcutaneous route into a normal monkey which also died with lesions of visceral yellow fever There was no mention of the pathogenicity of this virus for mice

Findlay and MacCallum (1938) on three occasions produced death with hepatic lesions in monkeys inoculated intracerebrally with the French neurotropic virus after 753 757 and 762 mouse brain passages respectively In two instances the infection was transmitted to other monkeys by the inoculation of blood intracerebrally Both animals died of yellow fever hepatitis As virus representing the 671st mouse brain passage of the French strain when inoculated intracerebrally produced death from encephalitis in 12 and 13 days these workers concluded that a change had occurred after the 671st mouse brain passage The changed virus behaved in mice like a typical neurotropic one but had nevertheless acquired the power of producing hepatic necrosis in rhesus monkeys It is significant that the altered virus retained its ability to produce liver necrosis in spite of nine consecutive passages in mouse brain

Van den Berghe (1940) reported death with liver lesions in a baboon *Papio jubilaeus* (=*Papio comatus comatus* E Geoffroy) inoculated intracerebrally with the 322d mouse brain passage of the French strain Passage of the virus from the liver of the baboon to a rhesus monkey by subcutaneous inoculation caused death with visceral lesions The disease in mice produced by the virus from the brain of the baboon was as judged by the time of death highly neurotropic whereas the virus isolated from the liver caused death at a time more typical of a viscerotropic strain In an attempt to repeat this observation van den Berghe inoculated a monkey of the same species intracerebrally with virus representing the 327th mouse brain passage This

animal died of encephalitis The virus recovered from the brain and liver was highly neurotropic for mice A monkey inoculated subcutaneously with the liver of the baboon remained well but developed antibodies to yellow fever

A somewhat similar observation was made in the Yellow Fever Laboratory in New York A rhesus monkey inoculated intracerebrally with a mixture of two samples of 17D vaccine was found dead with typical lesions of visceral yellow fever 3 days after inoculation Six additional monkeys were inoculated intracerebrally with the same two vaccines either alone or in combination All six animals responded with a febrile reaction characteristic of a nonfatal encephalitis due to the 17D virus

While it cannot be categorically denied that a neurotropic virus can be reconverted into a viscerotropic one there is considerable doubt about the justification of this conclusion in view of the known occurrence of accidental infections in rhesus monkeys It may be recalled here that Findlay and MacCallum themselves reported the occurrence of spontaneous fatal yellow fever in two rhesus monkeys that had been kept in two separate rooms in which no other monkey infected with yellow fever had been quartered for 3 and 6 months respectively

At the New York laboratory there was no hesitancy in concluding that the death of a monkey which appeared to be due to the conversion of the 17D strain into a highly viscerotropic one was in all probability the result of an accidental laboratory infection The behavior of the mutant virus reported by Findlay and MacCallum highly neurotropic in mice and viscerotropic in monkeys is very much like that of the artificial mixtures of neurotropic and viscerotropic strains which has been described in the discussion of the interference phenomenon

VARIATION OF STRAINS IN NATURE

The pathogenicity for experimental animals of different strains of yellow fever virus varies greatly It has already been pointed out that strains isolated in Africa are as a rule more pathogenic for rhesus monkeys than South American strains This difference however is not absolute The degree of virulence of a strain for rhesus monkeys is not correlated with its neurotropic affinity either positively or negatively as measured in mice The two highly viscerotropic African strains the French and the Asibi differ markedly in their pathogenicity for mouse brains The Asibi strain is comparatively

highly neurotropic and on passage in mice becomes fixed very rapidly whereas the French strain is relatively avirulent for mice and does not become fixed so quickly

It has been pointed out that the rate of fixation for mice is a characteristic of a virus strain Different strains isolated from the same epidemic have similar rates of fixation and as far as one can judge from the limited information available this applies to strains isolated in urban epidemics as well as those isolated from cases of jungle yellow fever

Laemmert (1944) reported on the pathogenicity of various strains of yellow fever virus for rhesus monkeys and marmosets The two African strains the Asibi and French both highly pathogenic for rhesus monkeys were relatively avirulent for the marmoset *Callithrix jacchus,* whereas all South American strains with one exception were relatively avirulent for the rhesus but produced a high mortality in *C jacchus* The high mortality in rhesus monkeys produced by the exceptional South American strain was based on observations on a total of only four monkeys all of which died A similar difference in pathogenicity was observed when representative strains from the two continents were tested in another species of marmoset *Leontocebus rosalia* All of 18 *L rosalia* inoculated with the two African strains lived whereas all of 14 inoculated with two South American strains died

Further evidence of strain variation was found by Laemmert (1946) in testing the susceptibility of South American marsupials Two species *Marmosa cinerea* and *Caluromys philander,* were resistant to the inoculation of the J 7 strain but were susceptible to the O C and the Asibi strains

Essentially similar results were obtained in Colombia with another species of marsupial the common opossum *Didelphis marsupialis* Of numerous virus strains studied in this animal only one the Chichimene produced an infection regularly Opossums which were apparently resistant to other strains showed circulating virus when inoculated with the Chichimene

These variations in pathogenicity of different virus strains are in all likelihood due to variation and selection occurring in nature during the various transmission cycles the virus undergoes

In transmission experiments with *A aegypti* and the marsupial *Metachirus nudicaudatus* Waddell and Taylor (1948) found differences in the facility with which cyclic passage could be maintained depending on the strain of virus used The Almada strain could be more readily transmitted than the O C strain However the latter became better adapted to cyclic passage in

metachirus after it had been transmitted several times in marmosets and metachirus by means of mosquitoes

THE RELATIONSHIP OF YELLOW FEVER VIRUS TO OTHER VIRUSES

The disease of man which most closely resembles yellow fever is dengue Both diseases may be transmitted in nature from man to man by *A aegypti* in both the blood of infected man is infectious for the mosquito only during the first 3 or 4 days of the disease Furthermore after taking up an infective feed of the virus of either disease mosquitoes are unable to transmit the disease until several days have elapsed. *Aedes albopictus* which has been incriminated as a vector of dengue in addition to *A aegypti* does not occur in regions where yellow fever is prevalent but it has been shown to be capable of transmitting yellow fever virus experimentally

Rhesus monkeys and mice are susceptible to both viruses In rhesus monkeys dengue virus produces a symptomless infection whereas yellow fever virus usually produces a more severe disease due probably to the more extensive involvement of the liver That the virus of dengue too has an affinity for the liver is suggested by the fact that it will interfere with yellow fever virus and protect a monkey from death By serial mouse brain passage both viruses become attenuated for man Yellow fever virus modified by mouse brain passage when inoculated into the nervous system of monkeys produces an encephalitis A dengue virus modified by serial mouse passage similarly acquires the ability to produce lesions in nervous tissue when inoculated intracerebrally into monkeys Complement fixation tests by Sabin (1949) have shown that the two viruses are related

A second virus that is strikingly similar to that of yellow fever is the virus of Rift Valley fever The lesions produced in the liver of animals infected with this virus resemble very much those produced by yellow fever virus Again both viruses are pathogenic for rhesus monkeys Moreover it has been pointed out that Rift Valley fever virus is able by interference to protect monkeys against death from yellow fever However it differs from yellow fever virus in its pathogenicity for mice producing a fatal infection in mice of all ages by parenteral inoculation death being due to hepatic necrosis Yet the fact that yellow fever virus is able to protect mice to some extent against the virus of Rift Valley fever is suggestive evidence that the virus of yellow fever too has an affinity for the liver cells of mice No experi

mental evidence is available on immunologic overlap between the two viruses, but it is known from accidental laboratory infections that persons immune to yellow fever are not immune to Rift Valley fever.

By the passage of Rift Valley fever virus intracerebrally in mice, MacKenzie, Findlay, and Stern (1936) and Smithburn (1949) obtained strains which have to a considerable extent lost their viscerotropism. Sheep inoculated subcutaneously with the attenuated mouse passage virus were rendered immune to a subsequent inoculation of a highly virulent strain. While the mode of transmission of Rift Valley fever has not been determined, epidemiologic evidence points to the mosquito as the vector. Smithburn, Haddow, and Gillett (1948) have reported the isolation of the virus of Rift Valley fever from several lots of *Eretmapodites* caught in a forest in Uganda, indicating not only that this genus of mosquito probably plays a part in transmission but also that there is a reservoir in the forest from which the infection might be disseminated. Just as there is jungle yellow fever, there appears to be also jungle Rift Valley fever. By transmission experiments Smithburn, Haddow, and Lumsden (1949) have shown that *Eretmapodites chrysogaster* can act as a vector of Rift Valley fever. This species of mosquito had previously been found by Bauer to be a vector of yellow fever under experimental conditions.

Of the diseases caused by the three viruses discussed so far, yellow fever and Rift Valley fever in man and domestic animals are due to the dissemination of the virus from some reservoir in nature. Whether or not this is true of dengue is not known. The possibility, however, that there is a similar reservoir of dengue has not been excluded. It may be that the aegypti transmitted dengue epidemics are, like those of yellow fever, a manifestation of the action of the virus when transmitted by an aberrant vector.

By complement fixation tests, evidence has been obtained (Sabin, 1949) that the virus of yellow fever is related not only to that of dengue, but also to the West Nile virus and the virus of Japanese B encephalitis. Dengue immune sera will fix complement in the presence of antigens prepared not only from the homologous virus, but also with antigens prepared from the Japanese B, West Nile and yellow fever viruses. A yellow fever immune serum will fix complement in the presence of antigens prepared from yellow fever, dengue, West Nile, and Japanese B viruses. Furthermore, it has been shown that the serum of an animal immunized to the West Nile virus will fix complement to West Nile, Japanese B, and St. Louis encephalitis viruses. Finally, a close relationship has been shown to exist between the

viruses of louping ill and Russian encephalitis. While the significance of the complement fixation test as applied to viruses is still a matter of debate and consequently deductions drawn on the basis of results of the test are open to question, nevertheless it is probable that the overlap does indicate some similarity in the viruses.

More definitive information has been given by the mouse protection test. It too has provided evidence of considerable overlap. It has shown that the West Nile, Japanese B, St. Louis encephalitis, and louping ill viruses are immunologically related (Smithburn, 1942). Furthermore, it has shown the viruses of Russian encephalitis and louping ill to be very similar (Casals, 1944).

All the viruses so far discussed have certain features in common. As far as has been determined, the particle size of all is approximately the same; all are probably transmitted in nature by arthropods, and all are pathogenic for mice by intracerebral inoculation. Infection of man or his domestic animals is probably in all cases an accidental one, a manifestation of the virus in an abnormal or aberrant environment. To the group mentioned above should be added the three immunologically distinct viruses which produce encephalitis in horses and man—the eastern, the western, and the Venezuelan equine encephalomyelitis viruses.

The types of disease produced by all of these viruses are similar to those produced in man or experimental animals by unmodified or modified strains of yellow fever virus. It is perhaps unfortunate that the term encephalitis has become attached to so many viruses. While it is true that the viruses of equine encephalitis and St. Louis encephalitis were first brought to the attention of scientists on account of the severe nervous involvement observed in infected humans and horses, it must be emphasized that this involvement is a relatively rare and accidental occurrence. The more common type of infection is usually systemic with only occasional involvement of the central nervous system. The disease produced in monkeys by a neurotropic yellow fever virus strain is very similar to the disease produced in man and domestic animals by natural infection with the various encephalitogenic viruses. Furthermore, *involvement of the central nervous system has been at* times observed in human cases of yellow fever contracted in nature.

Thus yellow fever virus is merely one of a very large group of viruses of approximately the same size whose pathogenic characteristics are merely modifications of one basic type. All have been shown to be transmitted in nature by arthropods with the exception of the West Nile virus, for which

no information is as yet available and all have distinct neurotropic affinities All probably evolved from a common ancestral type During evolution adaptations to various vertebrate and invertebrate hosts have occurred Of necessity a vertebrate host to be a source of infection for the blood sucking arthropod must undergo an infection characterized by the presence of virus *in the circulation* Infection of the nervous system must therefore be considered as a case of aberrant parasitism Neurotropism as such has no selective value and can thus be of no significance from an evolutionary point of view However the fact that all the viruses in the group under discussion do show neurotropic affinities must have some explanation While in general it may be stated that organisms become parasitic if they have lost the ability to synthesize essential substances that they can find only in other living organisms (their hosts) virus infections may be looked upon as extreme forms of parasitism and consequently dependent on the host cell almost entirely for the necessary materials for their own regeneration The *viruses under discussion find the necessary conditions on the one* hand in the arthropods and on the other hand in cells of the vertebrate hosts The knowledge available at the present time gives no indication of what specific biochemical environment is shared by certain cells of the arthropod and certain cells of the vertebrate host

The great plasticity of the virus of yellow fever has been pointed out Marked variations are induced by its propagation in different tissues of various animals That somewhat analogous variations occur *in nature* is demonstrated by the difference in pathogenicity for experimental animals shown by various strains This plasticity affords the opportunity for the virus to become adapted to various hosts vertebrate as well as invertebrate in nature In comparing the pathogenicity for experimental animals of strains isolated from South America and Africa suggestive differences have been discussed But the strains from both continents though somewhat different in pathogenicity are as far as can be determined *immunologically identical* A somewhat greater divergence has occurred in the evolution of dengue virus But although there are immunologic differences between strains of this virus isolated in different parts of the world there is still considerable antigenic overlap In the case of the eastern and western equine encephalomyelitis viruses the immunologic divergence is almost complete

3 THE PATHOLOGY OF YELLOW FEVER

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YELLOW FEVER in man and animals is an acute infection of varying characterized by rapid proliferation of the causative virus in vario cells The disease whose duration is to be measured in days te either in complete recovery and permanent immunity or in death of the abrupt onset and rapid course of the infection the patholog are acute in type and fundamentally degenerative in character Th are predominantly visceral the liver and kidneys are especially and are responsible for most of the clinical manifestations

In severe cases marked jaundice usually occurs as well as a hem tendency to which some of the more spectacular features of th may be attributed Hemorrhages into the stomach and intestinal tr the basis for both the black vomit (*vomito negr*) and the melena monly seen in fatal infections

Our knowledge of the pathology of yellow fever is based largely findings in fatal cases where the lesions are naturally most severe in the far more numerous nonfatal infections we can only infer th of the pathology Further there is a large proportion of very m where the clinical manifestations if present at all are usually h headache moderate fever of short duration and slight albumin cannot be expected that the histopathologic changes in such cases more than a mild cloudy swelling of the renal epithelium and slight ired congestion

With the more severe infections however the pathologic chan be profound with nearly every organ and tissue affected to some These cases have a high mortality and form the category most comm sidered in discussing the clinical and pathologic manifestations o fever

At one time it was thought that African and South America fever might be different diseases and that their pathologic pattern also be different The confusion of spirochetal jaundice with yell (Noguchi 1919*a*) for a time lent support to this theory and encour consideration of the disease geographically The work of Sawyer et al (1930) [illegible]

fever distributed widely over Africa and South America was a single disease.

The development of methods for the isolation and identification of yellow fever virus made possible the certain diagnosis of the disease and an exact study of its pathology. As a result, it was found that the pathologic changes were consistent in pattern and that they corresponded very well with the descriptions of Councilman (1890), to whom we owe the first reasonably complete description of the lesions of yellow fever, and with descriptions of later workers. In the early years of the twentieth century, Marchoux and Simond (1906*a*, *b*, and *c*) in Brazil had included pathologic studies in their broad attack on the problem of yellow fever; at about the same time Thomas (1910) of the Liverpool School of Tropical Medicine Expedition to Manaus had carried out similar pathologic studies. Nevertheless, while all of these workers described the general histopathology quite completely, they were not fully aware of the pathognomonic character of the liver lesion which later was so useful in the viscerotomy services of South America.

HUMAN PATHOLOGY

GENERAL APPEARANCES

Considering the violent course of fatal yellow fever, the general signs of damage to the body and the gross pathology as a whole are surprisingly unimpressive. There is commonly a moderate generalized icteric staining easily visible in the white person but difficult to detect in the African. In Africans there is frequently the additional complication of a yellow staining of the conjunctivae and subcutaneous tissues resulting from the use of palm oil in the diet.

Externally, in addition to the icterus, hypostasis is marked due to the greater fluidity of the blood. Small hemorrhages may occur into the skin and beneath the conjunctivae and mucous membranes. Free blood in the mouth from bleeding of the gums is common.

On opening the body cavities, the serous surfaces are moist and icteric with a slight to moderate increase in the amount of pleural and peritoneal fluids. In uncomplicated cases there is no exudate over any of the serosae. The liver is ordinarily slightly enlarged and exhibits a yellowish pallor.

The stomach may contain a considerable amount of partially digested blood that has exuded from a large number of petechial hemorrhages of the mucosa; small erosions may be found at times in association with these

hemorrhages. Such hemorrhagic changes also occur in the duodenum but usually do not involve the small intestine to any extent. However, the small intestine and the colon may contain a variable amount of partially digested blood that has descended from the stomach. In the bladder, submucosal hemorrhages are frequently found, and at times the small amount of urine is bloodstained.

The essential pathologic processes will be described systematically for the organs showing lesions of consequence.

HEART

Grossly the heart is somewhat flabby with a variable amount of subserous petechial hemorrhage and a moderate dilatation of the right ventricle. Icterus is often visible and may be moderately severe in some cases.

On section, the myocardium is cloudy, icteric, and soft, and there is some dilatation of all of the valve rings. Subendocardial hemorrhages may be present but are highly variable. More constant is a patchy subendocardial fatty change that may extend deeply into the myocardium.

Microscopically, there is almost always cloudy swelling and degenerative fatty infiltration of the muscle fibers (Otto and Neumann, 1905; da Rocha Lima, 1912a; Klotz, 1927; Cannell, 1928) with fine vacuoles of fat in a faintly granular sarcoplasm. The fatty change tends to be most severe immediately beneath the endocardium and is ordinarily uneven in distribution, producing the mottled appearance frequently seen upon gross examination. The nuclei of the muscle fibers tend to be enlarged and often show hydropic changes leading to actual necrosis with karyolysis and vacuolization of the cytoplasm.

These lesions may occur not only through the myocardium but also in the sinoauricular node and the bundle of His (Lloyd, 1931). In the two latter sites the changes are in accordance with the clinically observed bradycardia and alterations in the electrocardiogram. In experimental yellow fever in the rhesus monkey, a severe Zenker's necrosis of the fibers of the sinoauricular node at times accompanies the fatty changes and hydropic degeneration. Sometimes actual necrosis of the ganglion cells is encountered. In the studies of Lloyd, the severest lesions were associated with the greatest degree of bradycardia.

The pathology of the sinoauricular node also applies to the auriculoven

tricular bundle, the changes in the ventricular muscle generally are similar in character, the differences being those of degree and not of k

This involvement of the conduction system is of considerable clin portance, for bradycardia during convalescence is a common finding frequently associated with other evidence of myocardial insufficiency den death following exertion during convalescence has often been ob and is probably upon the basis of the myocardial damage.

In the heart, as in other structures, the yellow fever lesion is basi one of injury that is not followed by an effective inflammatory respo There is a striking lack of cellular infiltration and fibroblastic prolifera No permanent changes remain that can later be recognized with certain

AORTA AND OTHER GREAT VESSELS

While degenerative changes of the major blood vessels have been described in cases of yellow fever, the lesions must be considered simply as coincidental with the acute disease and not related to it. Other than the icteric staining and an occasional subintimal hemorrhage, there appears to be no significant pathologic change of these vessels due to yellow fever.

LUNGS

The alterations that the lungs may exhibit are incidental to the functional incapacity of other more immediately affected organs. Ordinarily there is a definite icteric tinting as part of the general bile staining. In the dependent portions of the lung the air content is reduced due to atelectasis and moderate edema. The entire lung is congested to a degree dependent chiefly upon the extent of the cardiac damage. As a consequence of the fluidity of the blood, the organ exsanguinates readily and may present a pale appearance with accentuation of the icteric tint.

Secondary pathology, such as that of bronchopneumonia, tuberculosis and other pulmonary diseases, may be encountered with considerable frequency. However, these conditions are not properly a part of the pathology of yellow fever.

LIVER

The most characteristic of the lesions produced by the virus of yellow ver are found in the liver, although hepatic failure need not be the

mary cause of death (Fig 22) The presence of jaundice especially in th fatal cases has always drawn attention to the liver so that hepatic patholog has been stressed The general description of the liver lesion by Council man (1890) was an accurate one that merited greater attention than it received at the time Later the studies of Marchoux and Simond (1906a b and c) in Brazil and those of da Rocha Lima (1912a 1926) Magarinos Torres (1926) Klotz and Simpson (1927) Cowdry and Kitchen (1929 1930) and Klotz and Belt (1930a c and d) all contributed to the advancement of the knowledge of liver pathology in yellow fever

Gross Pathology The changes in the liver visible to the naked eye are not striking and rarely prepare one for the degree of damage that may be visible under the microscope There is but little deviation in volume from the normal and the spectacular reduction in size occurring in typical acute yel low atrophy is never found Frequently the organ appears moderately nlarged a change due as much to the passive congestion as to the parenchy matous damage The color is highly variable in the extreme cases bein reddish yellow changing to a more definite yellow as the blood is allowed to drain out Under these conditions the appearance of the liver is often described by the term boxwood

On section the cut surface may exhibit intergrading yellow and pink colors depending upon the relative amount of congestion and icterus pres ent The lobular markings are indistinct and the entire cut surface has a smooth and featureless quality due to the diffuseness of the degenerative changes The tendency of the liver parenchyma to protrude at the cut edges is indicative of the cloudy swelling and the larger portal vessels are sunken below the general plane of the cut surface The coloration results from a combination of bile pigments and the marked diffuse fatty changes that invariably occur thus the cut surface may vary not only in color but also in the degree of greasiness

No vascular changes occur other than those resulting from bile staining Often the gall bladder is distended with a heavy highly pigmented bile and occasionally there are subserous petechial hemorrhages otherwise here is no change in the biliary system

Microscopic Pathology Only when the tissue is examined in microscopic ction can the extent of the damage to the liver be appreciated Prompt rmol fixation followed by sectioning and staining with hematoxylin and sin reveals a very diffuse disorganization of the parenchyma In the typi case the changes are most marked in the midzone of the liver lobule

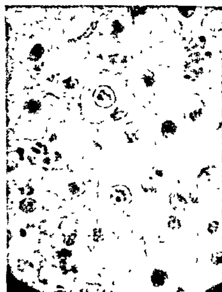
with extension of the process both centrally and peripherally T
lesion is a necrosis that is discontinuous in the cords of liver cells
sents the termination of a series of changes that begins with cloudy
and progresses through degenerative fatty infiltration to a simple and
lative necrosis

The early changes in the liver cells are those of any toxic injury ar
manifested by a turgescence of the entire cell including the nucleus
pronounced granularity of the cytoplasm Then comes the formation of
fat both within the nucleus and in the cytoplasm The extent of fat for
tion depends largely upon the location of the injured cell in the lob
and upon other physiologic factors The fat is a mixture of large and sm
droplets and appears to have a complex origin Some of the lipid in larg
globules results from the excess of intake over conversion of the fat but mos
of the material seen in fat stains is composed of glycerol esters in small
droplets whose accumulation is part of the degenerative lesion of the liver
cell This degenerative fatty infiltration is associated with the general dam
age to the cell protoplasm as a result of which the normal enzymic activity
is reduced or suppressed The small droplets of lipid are exceedingly numer
ous throughout the lobule although they are usually more abundant in the
central and intermediate zones than peripherally

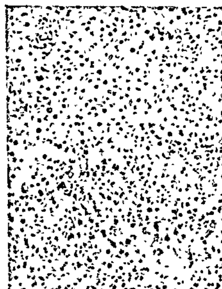
Where the damage is greatest the cells pass into a more profound necro-
biotic phase with migration of chromatin to the nuclear membrane followed
by rupture of the membrane and scattering of the nuclear fragments The
entire cell content may then undergo an intracellular coagulation resulting
in a discrete hyaline cohesive mass with a marked affinity for eosin The
masses or bodies resulting from this intracellular coagulation necrosis were
originally described by Councilman (1890) and are commonly known as
Councilman bodies In some instances the nuclear structure persists some
what and may be faintly seen in the larger eosinophilic body

Typically the involvement of the liver cord is discontinuous so that liv
ing cells are interspersed with necrobiotic ones to produce a random arrange-
ment often described as pepper and salt (Magarinos Torres 1926) While the
ntermediate zone is characteristically more profoundly affected the coagu
ative necrosis may be found throughout all portions of the lobule In the
ore fulminating cases of yellow fever the necrosis of the midzone may be
me nearly confluent and the more typical picture will then be visible
y in the zones less intensely affected

ven with the most advanced necrosis the cells immediately bordering


(a)


(b)


(c)

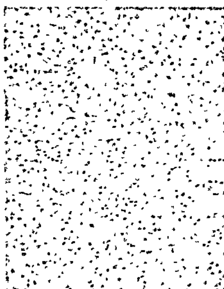

(d)

FIG 22 Human and monkey livers in yellow fever (*a*) Rhesus monkey liver in fatal yellow fever Swollen degenerating cell nuclei showing nuclear inclusions ×1,000 (*b*) Rhesus monkey liver in fatal yellow fever ×80 (*c*) Human liver showing necrotizing lesion of yellow fever Necrosis and Councilman body formation most marked in midzone ×250 (*d*) Rhesus monkey liver in fatal yellow fever, showing surviving liver cells about central vein ×250

upon the central vein are spared for the most part In contrast with hepatic diseases in which the central zone is most severely involved there is always a collar of surviving cells about the central vein a characteristic that has a certain diagnostic value in yellow fever

With the collapse of the liver cords the integrity of the bile canaliculi is lost *and the normal metabolism and formation of bilirubin is interrupted* A variable amount of diffuse bile staining occurs with considerable bile pigment in the reticuloendothelial cells but there is no obstruction of the bile passages and no distension of those bile canaliculi that remain functional

The blood vascular system normally remains intact so that hemorrhage is not characteristic of the liver lesion although in some instances small extravasations of red blood cells may be encountered

While the Kupffer cells do not appear to be outstandingly affected they are swollen and cloudy and often contain considerable quantities of bile pigment Small amounts of iron containing pigment (presumably hemosiderin) are frequently found occasionally fat droplets are prominent The Kupffer cells not only are enlarged but in many instances are increased in number especially where the process has been less severe than in the usual fatal case Where the disease is fulminant the reticuloendothelial cell nuclei may be pyknotic and show considerable karyorrhexis

It is probable that in the human liver the depletion of glycogen shown experimentally *in the rhesus monkey* is an important part of the pathology In the early phase of injury glycogen is lost rapidly and by the time necrosis is established most of the glycogen has disappeared In general the diminution of glycogen is proportional to the intensity of the liver injury (Klotz and Belt 1930*a*, *c* and *d*)

In the series of 91 cases studied by Klotz and Belt the proportion of the liver lobule involved in the necrotic process varied from 5 to 100 per cent with an average of about 60 per cent The distribution of the necrotic process was not uniform in many of the livers and in some there was a marked difference between the left and right lobes This phenomenon has also been observed by the author It is probably related to the differing degrees of congestion in the two lobes caused by pressure *of the round ligament* on branches of the hepatic vein In one case this pressure was so marked as to produce a unilateral appearance of severe chronic passive congestion

An outstanding feature of the entire extensive pathologic process in the liver is the absence of significant inflammatory reaction There is no important cellular response to the yellow fever necrosis itself Especially in

African patients antecedent hepatitis may produce a liver in which there are extensive round cell infiltrates but these have no relation to the superimposed yellow fever lesion The characteristic lack of inflammatory reaction in the liver is not unique this nonresponsiveness extends to the other tissue elements as well There is no fibroblastic proliferation about the necrotic cells as is usual following liver damage from other causes nor is there any evidence of the proliferation of bile duct epithelium

Nuclear Changes The nuclei of the surviving liver cells may also undergo a characteristic change in yellow fever Magarinos Torres (1928*a b c d* and *e*) recognized the similarity of the intranuclear eosinophilic bodies previously seen by Stokes Bauer and Hudson (1928*b*) and by Hindle (1929) to the inclusion bodies of herpes virus infections He found that these acidophilic granules were nearly always present in the livers of rhesus monkeys and were present with less frequency in human specimens

A detailed study of these inclusion bodies was made by Cowdry and Kitchen (1929 1930) who utilized liver tissue from rhesus monkeys experimentally infected and from human cases In the monkeys the inclusions were not found until the first day of fever after which they were encountered regularly In the human material the inclusion bodies although similar in form to those seen in the monkey livers were present relatively infrequently The bodies are formed through the development of discrete clumps of acidophilic particles within the nucleus the clumps increase in size until they occupy most of the space within the nuclear membrane During this period the nucleolus maintains its central position but the basophilic chromatin comes to lie against the nuclear membrane In later stages Cowdry and Kitchen found that the inclusions become more compact the nucleolus disappears and the total volume of the nucleus diminishes In some instances the basophilic chromatin may disappear completely and the nuclear membrane may rupture leaving the inclusions lying free

That these bodies were neither artifacts nor the result of the action of fixatives was demonstrated by Cowdry and Kitchen by means of dark field microscopy of fresh tissue This technique permitted them to visualize the bodies with ease After comparing the bodies with those induced by other viruses Cowdry and Kitchen concluded that the form of the inclusion is characteristic of yellow fever and that such bodies when well displayed are diagnostic of the disease

Nothing is known of the virus content of these inclusion bodies but it seems highly probable that they are the result of degenerative changes

within the cell nucleus and do not represent actual virus as some investigators have suggested in the case of other types of inclusions While the yellow fever inclusion bodies have a distinctive form and character they do not appear to differ in their substance from the remainder of the nucleus As a matter of fact the name inclusion body may be somewhat misleading in that the bodies do not represent material foreign to the cell but are in reality derived from it

Pathology of the Liver in Delayed Death The studies of Villela (1941) threw considerable light upon the problem of the pathologic diagnosis of yellow fever in cases where death is delayed His material consisted of a series of specimens where the clinical diagnosis had not been supported by the pathologic findings (In one instance however the clinical diagnosis had been confirmed by the isolation of virus on the 2d day of illness 9 days before death) Review of this material disclosed certain distinctive histologic changes which could be associated with the fact that the specimens came from patients who had died much later in the course of the disease than usual and in some cases of causes not directly connected with yellow fever

Councilman bodies could not be found in most of these specimens but in some it was possible to trace a continuity of change from typical Councilman bodies through a process of shrinkage and absorption of bile pigments to small irregular and granular ocher-colored bodies visible chiefly in the midzone These Villela bodies represent an intermediate stage in the complete disintegration of the Councilman bodies Since the end picture in which Villela bodies are found is strikingly dissimilar to the classic appearance of the liver in yellow fever the presence of these bodies appears to lend a high order of validity to the diagnosis of the late stage of the disease

Subsequent study of similar material by Smithburn led him to the conclusion that a considerable proportion of the ocher bodies are in reality Kupffer cells actively engaged in disposal of the cell debris

In addition to its diagnostic value Villela s work has thrown light on the mode of recovery of the liver from yellow fever for he observed that throughout the entire succession of changes from Councilman body to Villela body there was no inflammatory reaction to the necrotic cells nor was there any striking proliferation of liver cells to replace those destroyed by the virus

Healing of the Liver Following Yellow Fever It has been common knowledge for many years that the livers of persons who have experienced clinical yellow fever several years before death exhibit no changes that can with certainty be attributed to the disease The presumption of effective regenera

tion was tested by Klotz and Belt (1930*d*) who studied the livers of a number of rhesus monkeys that had recovered from definite attacks of yellow fever The animals were killed at periods ranging from 16 to 72 days following the subsidence of fever and no changes were found that could be ascribed to the virus Consequently Klotz and Belt concluded that regeneration of liver cells is rapid and complete so that the liver is restored to normal by the end of convalescence without any proliferation of stroma or any of the other changes commonly encountered in the reparative activities of a damaged liver They considered the findings a striking illustration of Mallory s (1911) contention that destruction of the parenchymal cells does not of itself stimulate connective tissue proliferation and that there must be some degree of irritation of the stroma before fibroblastic activity will ensue Since the necrosis in yellow fever is a coagulative one there is no proteolytic activity neither is there any action upon the stroma which later serves to guide new cells into the cord arrangement

While the conclusions of Klotz and Belt appear to be in harmony with the pathologic findings in man as well as in the rhesus monkey it should be realized that in the animals they studied there was no evidence that there had ever been any necrosis All they really showed was that the monkeys failing to die of yellow fever had normal livers when examined 16 or more days later Theiler found from repeated biopsies of the liver that rhesus monkeys inoculated with the neurotropic and J W strains of yellow fever virus recovered without ever showing any necrotizing lesion

Even though the evidence for man is not complete the work of Villela with human cases mentioned above supports the general conclusions very well Little doubt remains that in recovery from severe yellow fever a prompt effective regeneration combined with hypertrophy of the surviving cells takes place at a rapid rate There is nothing to indicate that the liver of the yellow fever convalescent is permanently damaged in any respect

SPLEEN

Gross examination of the spleen from a human being who has succumbed to yellow fever seldom reveals striking changes The organ may be slightly enlarged in uncomplicated cases but since most instances of yellow fever to day occur in the tropics where malaria is also very prevalent marked splenomegaly is a frequent finding although it is not intimately related to the

yellow fever The capsule is smooth the color dark and on section the parenchyma is found to be friable

Microscopically however there is a profound alteration in the spleen in yellow fever The changes are useful in diagnosis although they are by no means as obvious or as characteristic as those in the liver The sequence of these tissue modifications was carefully studied by Klotz and Belt (1930*b*) in spleens from both human patients and rhesus monkeys The first observed change is the appearance of large mononuclear cells in and about the follicle As the mononuclear cells increase in number the follicle may be distended and its boundaries partially obscured The cells are nearly round with a diameter of about 15 micra The cytoplasm is somewhat basophilic and clear The nucleus is relatively large with a smoothly irregular surface often with several large nucleoli there is a moderate amount of deeply staining chromatin These cells are not phagocytic and were thought by Klotz to arise in situ so that he referred to them as primitive mononuclears In any case they appear to be closely related to the lymphoid structure

As the large mononuclear cells increase in number there is a progressive diminution in the number of lymphocytes culminating in an inversion of the ratio between the two cell types Still later the mononuclears also diminish and there is a marked reticuloendothelial hyperplasia so that the follicular structure becomes replaced by a rich growth of small ovoid cells joined by delicate cytoplasmic processes Thereafter within such hyperplastic tissue new germ centers may appear but they commonly degenerate

In an appreciable proportion of fatal cases large multinucleate cells of the bone marrow type may be found in the spleen These are not peculiar to yellow fever but are present in many conditions which involve a severe strain on the lymphoid structures

KIDNEYS

Although the liver lesion has long attracted the greatest attention in yel low fever because of its striking microscopic character and because of the presence of the accompanying jaundice it has also been recognized that idney function is profoundly affected While the renal lesion is not so dis nctive and diagnostic as that of the liver it probably plays fully as im rtant a part in causing death Albuminuria is one of the earliest signs of low fever and usually makes its appearance soon after the onset of fever apidly increases in intensity until in the severe cases the urine may co

agulate completely on boiling The onset of anuria is one of the signs of gravest prognostic import and is rarely followed by recovery

Grossly the kidney in fatal yellow fever is plump and turgid with a variable amount of icterus visible from without the capsule Occasionally small subcapsular hemorrhages are seen as a part of the general tendency to hemorrhage On section the kidney substance is clouded swollen and gray pink in color with a variable amount of yellow streaking in the medullary rays and portions of the cortex

On microscopic examination following prompt fixation the glomeruli show fewer changes than the tubules The capillary tuft is usually moderately engorged the capsular space somewhat distended and the cells lining Bowman s capsule show varying degrees of degeneration from cloudy swelling and desquamation to simple necrosis Small globules of fat are encountered in the capsular epithelium and at times about the capillary tufts

The tubular epithelium shows severe changes especially in the convoluted tubules both proximal and distal All of the epithelial cells exhibit cloudy swelling and in some portions this is so severe that it merges into necrosis The necrotic process is ordinarily not massive and it tends to be discontinuous in a way reminiscent of the pathology of the liver With these alterations there is a variable amount of degenerative fatty infiltration which affects all portions of the tubular system in an irregular manner The fat droplets are found toward the bases of the cells and occur in both the convoluted and straight portions

The lumina of many or most tubules are filled with granular debris derived from the epithelial cell necrosis Hyaline and granular casts are seen in the collecting tubules Within Henle s loops especially but also in the *convoluted portions* calcareous masses showing a certain amount of structure are frequently encountered Not only do the masses contain calcium salts but Magalhaes (1931) in his excellent work on the pathology of the *kidneys in yellow fever* was able to demonstrate an appreciable iron content This type of cast is quite different from the calcareous changes found in mercuric chloride poisoning and its presence has been suggested as a *diagnostic aid in yellow fever*

Renal congestion is part of the general manifestation of the terminal vascular collapse Rarely hemorrhage into the glomerular space may occur

Magalhaes also reported finding acidophilic bodies within the nuclei of the tubular epithelium especially in the convoluted portions These did not have the granular and irregular character of the inclusions in the liver

described by Magarinos Torres and by Cowdry and Kitchen and appare were the result of a somewhat different process True inclusion boc were not found in the kidney by Cowdry and Kitchen and it is probal that Magalhaes was correct in thinking that the bodies he saw were necr biotic nucleoli

In this connection it is to be remembered that cytologic studies on human tissues taken at autopsy must be interpreted with caution Not only may ante mortem changes be markedly modified during even a short dela between death and fixation but intracellular and especially intranuclear changes may occur that are purely post mortem alterations having no relation to the true pathologic manifestations In the author s opinion aci dophilic granules or masses within the nuclei of cells of any organ should be considered as artifacts unless control tissues subjected to similar condi tions and manipulation consistently fail to show such structures Especially with respect to human tissues taken in the routine operation of a viscerotomy service must reports of inclusion bodies in either liver or kidney be received with skepticism

In the later phases of yellow fever there may be appreciable bile staining of the tubular epithelium as well as the formation of bile casts These latter are never so striking as in cases of obstructive jaundice when they may be visible to the naked eye

As in the liver despite the severity of the parenchymal lesion there is a striking lack of stromal reaction No fibroblasts appear irrespective of the amount of epithelial necrosis and there is no cellular infiltration to any important degree Necrotic cells disintegrate and their debris is dis charged into the lumina of the tubules making tissue digestion and ab sorption unnecessary In patients who recover it is probable that the ex tent of actual necrosis is small and that the defects are remedied through hypertrophy of the surviving cells plus some regeneration by direct multi plication

Whatever the degree of the renal lesion there is no subsequent fibrosis or vascular change and the kidney returns to a normal histologic appear ance Undoubtedly much of the descriptive pathology of yellow fever in the older literature is based on cases in which the diagnosis is questionable or in which unrelated pathology has been included with that of yellow fever In a population with an appreciable frequency of vascular disease it is inevitable that a large fraction of the individuals dying from yellow fever will exhibit pathologic vascular changes at autopsy Furthermore

when one recalls the difficulties inherent in the clinical diagnosis of yellow fever even today with the aid of specialized virus techniques and immunologic methods the past confusion as to the pathology of the disease is understandable

ADRENALS

Comparatively little has been recorded of the pathology of the adrenal glands in yellow fever Marchoux and Simond (1906*a*, *b*, and *c*) were impressed by the amount of lipid that they found in the fascicular zone of the adrenal cortex they considered the lipid to be associated with degenerative changes in the parenchymal cells because of the light staining of the cytoplasm the vacuolization of the nucleus and the migration of chromatin to the nuclear periphery The illustration included in one of the papers of Marchoux and Simond (1906*a*) would seem to indicate that there was an appreciable necrosis of the cortical cells of the outer fascicular zone extending into the glomerular zone That this may occur in the adrenals of both man and rhesus monkeys was confirmed by Hudson (1928) Apparently the changes are due not to an alteration in the total amount of cortical lipid but to a shift in its distribution and in the character of the lipid masses

The medulla in fatal cases of human yellow fever so frequently shows advanced autolysis that assessment of the ante mortem pathologic changes is difficult However clouding of the cells together with a variable amount of fatty change does occur

STOMACH

The gross changes in the stomach produced by yellow fever are rather striking as seen in autopsy material But it is usually the quantity of partially digested blood within the cavity of the organ that has been impressive rather than the mucosal lesions The amount of free blood lost by way of the stomach through persistent vomiting and passage down the intestinal tract may be considerable On careful examination of the mucosa large numbers of petechial hemorrhages may be seen From these there occurs a steady oozing of blood to the free surface often facilitated by the erosion of the overlying gastric mucosa About such erosions there may be infiltrations of polymorphonuclear leukocytes chiefly neutrophils and lymphocytes Such reactions follow any break in the mucosal continuity and are

not part of the essential pathology of yellow fever which in the case of the stomach is limited to the hemorrhagic tendency itself

SMALL INTESTINE AND COLON

The hemorrhagic process seen in the stomach may extend to the duodenum and at times to the beginning of the jejunum The lesions produced are of the same character Usually very little hemorrhage occurs below the level of the duodenum although the amount of free blood may be large As the blood moves down the intestinal canal it undergoes more complete digestion and becomes steadily darker until the mass is nearly black by the time it enters the colon Both the vomiting of blood and the melena thus have a common origin high in the intestinal tract Primary hemorrhage into the colonic mucosa is rare

The process described in the spleen also occurs throughout the lymphoid tissues of the intestinal tract itself There is a proliferation of large round cells with a diminution of mature lymphocytes The large cells have little or no phagocytic power and late in the disease they begin to disappear Germ centers are diffuse or are not visible at all

The widespread change in the lymphoid tissues apparently is associated with the constant lymphocytopenia encountered in yellow fever The type of cell that proliferates in the lymphoid tissues is fixed and does not escape with any great frequency into the circulation As in the spleen this cell would seem to be a primitive member of the lymphocyte series whose maturation is prevented by the disease process

A moderate amount of virus can usually be recovered from the lymphoid tissues even though there is little or no circulating virus in the blood stream It seems that there is actually some multiplication of the infectious agent throughout the reticuloendothelial system generally but the amounts produced tend to be far less than can be recovered from the liver and in experimental animals from the nervous tissues

PANCREAS

Considerable bile staining and patchy hemorrhage of the pancreas are usual findings in human yellow fever Microscopically there may be diffuse extravasation of red cells into the stroma of the organ Associated with this is a variable amount of fatty change manifested by small globules

of lipid toward the basal portions of the acini The differentiation between the acinal and centroacinal cells may be lost (Marchoux and Simond 1906a) and the islands of Langerhans may also partake in the degenerative fatty change Actual necrosis of the secretory cells as a result of virus action has not been reported and most of the changes encountered may be attributed to the profound vascular endothelial alteration and to the hemorrhagic propensity

BRAIN

One of the surprising aspects of yellow fever from the clinical point of view is the relative mental clarity of the patient even though he may actually be near or at the point of death This was dramatically recorded by Lundie (West Africa Yellow Fever Commission 1916) in his account of his own bout with the disease in the Gold Coast It might be expected therefore that the lesions of the brain and central nervous system in human yellow fever would be minimal in character

Fourteen cases of yellow fever in man were studied with respect to the pathology of the central nervous system by Jakob Tralho and Villela (1929) who reported that they found macrophages containing blood pigment and round-cell infiltration in the leptomeninges They also noted severe fatty changes and chromatolysis in the nerve cells themselves and proliferation of glia

This problem was further investigated by Stevenson (1939) using material from proved cases of yellow fever in Brazil and Colombia A preliminary study of small pieces of 11 brains had indicated that a more comprehensive inquiry would be justified whole brains from 20 cases were therefore obtained and these constituted the subject material of the researches reported

Grossly no abnormality was visible except for a small number of hemorrhagic spots on a few occasions In some instances the prosector had noted that the cerebrum had been congested There was opacity of the cerebrospinal fluid in six instances

The one consistent microscopic finding was small perivascular hemorrhages These were usually confined to the spaces about small arteries and veins and often about capillaries so that the involved vessels showed cuffs of red cells In such regions a moderate edema was encountered but there were no significant cellular infiltrations whatever round cells were present were in the main those incidental to the hemorrhage In nine of the 20

specimens there were occasional small lymphocyte infiltrations which were not clearly related to the acute process

Although the small hemorrhages were widespread they were present in concentration in the mammillary bodies and in the optic thalamus In this region they usually occurred near the lining of the third ventricle and about some of them there was a slight glial proliferation No evidence of demyelinization was found in any instance nor were there changes in the nerve cells other than those encountered in any toxemia

Stevenson concluded that the involvement of the brain in naturally ac quired human yellow fever is minimal that it is related to the general hemorrhagic tendency and that there is no true encephalitis His findings are adequate to explain most of the neurologic complications of yellow fever such as the ptosis of the eyelid and the partial facial paralysis re ported by Findlay and Stern (1935)

Although in most cases the cerebral pathology is minimal Stevenson considered that in some instances the perivascular hemorrhage alone may cause death He stated As I have noted the hemorrhagic condition in the brain and spinal cord in yellow fever resembles very much what my associates and I and what others have seen in alcoholic encephalopathy However in yellow fever the condition is more severe and probably would be fatal even if no other lesions were present in the liver or other organs

While these studies leave little doubt as to the nature of the brain pathology in the usual type of human yellow fever it should be remem bered that the neurotropic strains of virus often produce severe and some times fatal encephalitis in monkeys It is probable that the introduction of neurotropic virus strains into the central nervous system of man would be followed by similar results although studies on such cases have not been reported

MUCOUS MEMBRANES AND SKIN

The pathology of the covering tissues in human yellow fever is limited to the evidences of icterus and hemorrhage Submucosal and subepithelial petechial hemorrhages are common and the gums and mucous membrane of the mouth show *persistent hemorrhage* following slight trauma or even spontaneously At times subconjunctival hemorrhages are to be found Hemorrhage into the skin and mucous membranes occurs frequently in the more severe cases although it does not necessarily denote a fatal prog nosis

The pathology of human yellow fever has been sufficiently stud
permit separation of the lesions of the disease from those of antec
and unrelated processes Much of the former confusion was due to
of precise diagnosis so that various other acute infectious diseases
mistakenly classified as yellow fever and the essential pathology of yel
fever was obscured

The yellow fever lesion in whatever organ it is present has one ou
standing characteristic it is a selective necrobiosis that attacks only highl
specialized epithelial or myocardial cells The changes are fundamentally
toxic in character beginning with cloudy swelling and continuing through
degenerative fatty changes to a necrosis that because of the lack of pro-
teolysis is coagulative Stroma cells are not involved and there is no in
flammatory response to the necrosis either as cellular infiltration or
supporting tissue proliferation The prognosis depends in part upon the
extent of the necrosis and in part on the degree of injury to the surviving
tissues The most significantly involved organs are the liver and kidneys
Death results from the physiologic incapacity of one or both although
at times cardiac damage may contribute secondarily to the fatal outcome

While the renal lesion may be as important clinically as that of the liver it
is the hepatic process that is uniquely characteristic of the disease and that
can be recognized with certainty Basically the liver lesion is a discontinu
ous coagulative necrosis affecting primarily the intermediate zone of the
liver lobule and extending variably into the other two zones The necrosis
is discontinuous in the sense that it skips cells in the hepatic lobular cord
so that compact acidophilic masses representing necrotic cells are inter
calated with surviving ones The cells immediately about the central vein
always show less involvement than those in any other region and even in
he most severe cases there is a collar of surviving cells in this area The
tty changes are highly variable and are not in themselves diagnostic of
e disease although they are invariably present

Convalescent patients absorb the necrotic material slowly and show
plete replacement of the lost tissue by direct regeneration and hyper
hy of surviving cells There is no proliferation of stroma nor any other
ge leading to permanent incapacity of the organs involved
ondary to the liver damage and the resulting icterus is a widespread

tendency to hemorrhage which may reveal itself in widely diverse tissues and organs and which may give rise to a variety of clinical manifestations. Encephalitis is not part of the picture of naturally occurring yellow fever in man but presumably the introduction of an appropriate strain of virus directly into the central nervous system would be followed by encephalitis similar to that found in rhesus monkeys and other animals.

The recognition of the diagnostic value of the hepatic lesion led to the development of the viscerotome (Rickard, 1931), an instrument designed especially for quickly and easily removing liver specimens from persons dead of febrile disease. It also prompted the organization of a special service in Brazil (Rickard, 1937) for the systematic collection of such specimens. The work of this service defined the distribution of yellow fever among the human population and served to orient both control measures and epidemiologic research (Soper, Rickard, and Crawford, 1934).

PATHOLOGY OF YELLOW FEVER IN THE MONKEY

THE RHESUS MONKEY

The details of the pathology of yellow fever in the rhesus monkey were described by Hudson (1928), who brought out the similarities and differences between the rhesus and the human pathology. The process in the rhesus monkey is a fulminating one with exceedingly high virus titers. Probably as a result of this severity, the lesions as a rule tend to be more diffuse than in human beings. While the liver necrosis displays a midzonal selectivity, this is not as prominent as in man. There is much more diffuse extension to the other zones, leading to a massive necrosis involving most of the liver tissue. Even with this extensive involvement, however, the essential pattern is not lost, and in all zones surviving liver cells are found interspersed with coagulated masses representing dead cells. Probably because of the violence and speed of the disease in the rhesus, typical Councilman bodies are not formed to as great an extent as in human livers, but the fundamentally coagulative character of the necrosis is the same (Figs. 23 and 24).

Again because of the rapid course of the disease, icterus usually does not attain the intensity occurring in many human patients, although the general tendency to hemorrhage may be well developed. Bleeding of the

the howler monkeys which show an appreciable mortality depending upon the virus strain and the particular species The hepatic pathology of this group may simulate the human picture more closely than any of the others At the opposite extreme from the *Aotinae* are the species that rarely show any tissue reaction even though considerable quantities of virus may circulate in their blood Most of the African species come in this category as do some of the South American ones particularly the members of the genus *Cebus*

PATHOLOGY OF THE WHITE MOUSE IN YELLOW FEVER VIRUS INFECTION

The pathology produced by various strains of yellow fever virus in the white mouse was investigated by Theiler (1931) and by Goodpasture (1932) In both immature and adult mice the essential changes take place in the central nervous system which is also the chief site of virus multiplication

BABY MOUSE

At the time when baby mice are visibly ill with yellow fever there is a huge amount of virus in the brain Microscopically the nerve cells and neuroglia show widespread necrosis which may be either diffuse or focal In the surviving cells degenerative intranuclear changes occur the most conspicuous being the formation of amorphous finely granular acidophilic masses associated with irregular granules of basophilic material In the surviving cells of the basal ganglia these masses have the appearance of definite nuclear inclusions There is no diffuse cellular exudate in any portion of the central nervous system

ADULT MOUSE

Although the distribution of the lesions resembles that in the baby mouse the process in the older animal is a true encephalitis Widespread patchy necrosis involving both the neuroglia and the specialized nerve cells is accentuated about the basal ganglia where there also is found a marked perivascular round cell infiltration extending along the vessels into the leptomeninges

Where the mouse adapted neurotropic strain of yellow fever virus is

used, the process proceeds to a fatal termination so rapidly that there is no proliferative reaction With strains such as 17D, however which produce a disease of slower course proliferation of astrocytes may be found

The nuclear changes are variable In the nerve cells the lesions are all degenerative and may vary from simple swelling with hydropic change to pyknosis with karyolysis and chromatolysis Nuclear inclusions are not constant but occur most frequently in the region of Ammon's horn, where they are seen in Giemsa stains as irregular acidophilic masses usually surrounded by clear zones The number of inclusions frequently appears to be inversely related to the degree of cellular infiltration of the region

PATHOLOGY OF THE CHICK EMBRYO IN YELLOW FEVER VIRUS INFECTION

The chick embryo has been of inestimable value in yellow fever studies especially in the cultivation of virus strains and in the mass production of material for human immunization Although an embryo may contain an immense quantity of virus within 4 days after inoculation there is surprisingly little cellular reaction to the infection Only shortly preceding death does necrosis make its appearance The chief manifestation of infection in the chick embryo is simple congestion

Various local lesions have been observed and at times have been considered due to the virus However, Johnson has recently shown that the etiologic agent of fowl leukosis has undoubtedly been present in embryos used for yellow fever studies and that some if not most of the visible lesions were due to the unsuspected presence of that organism The matter will require restudy in the light of this recent development

4 IMMUNOLOGY

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THE IMPORTANT immunologic fact that an attack of yellow fever is followed by a solid immunity against reinfection was well known to the medical profession before Reed and his collaborators found that the causative agent of the disease is filtrable (Reed and Carroll 1902) Moreover long before any animal was known to be susceptible to yellow fever virus and thus suitable for laboratory studies Marchoux Salimbeni and Simond (1903) showed that the immunity resulting from an attack of the disease is associated with the development in the serum of substances capable of conveying passive protection in man The discovery of the susceptibility of the rhesus monkey to yellow fever virus by the West Africa Yellow Fever Commission reported by Stokes Bauer and Hudson (1928*b*) which enabled these workers to develop a highly specific test for immunity against the disease paved the way for intensive immunologic and other investigations The finding of Theiler (1930*a* and *b*) that certain strains of white mice are highly susceptible to the intracerebral introduction of the virus afforded an inexpensive host for laboratory studies and made possible the world wide surveys of immunity that were undertaken shortly thereafter The magnitude of these surveys and the many other uses that were found for the employment of immunologic techniques in yellow fever investigations brought numbers of workers into this field The resulting technical studies and the application of immunologic methods to epidemiologic investigations have probably exceeded in scope similar work on any other virus disease The outstanding immunologic investigations made since experimental hosts for yellow fever virus were discovered will be summarized in the following pages

In discussions of yellow fever and other virus diseases certain terms have in the past been more loosely used than will be the practice here For example in describing the difference between the reaction of members of the black and white races to yellow fever the milder reaction of the former is not infrequently ascribed to immunity—a usage to which the writer does not adhere In order to facilitate a clear understanding of the subject under discussion the usage of certain terms pertinent to the subject will be defined Susceptibility is that state of the tissues of the host that permits multiplication of the invading organism (virus) within them Immunity

is the altered state of the susceptible host resulting from previou ence with the invading organism (virus) by virtue of which it bec susceptible or less susceptible Active immunity is the result of an ence of the host itself with the virus Passive immunity is the re transfer to another host of antibody from a previously immunized Resistance is the innate ability of the host to prevent or to limit multi tion of the virus within its own cells Virulence is the capacity of a vading organism to damage the tissues of the host it is measured by degree and amount of tissue damage within a period of time and is ciated with the multiplication of the pathogenic agent

MANIFESTATIONS OF IMMUNITY IN YELLOW FEVER

The foremost manifestation of immunity to yellow fever is insuscepti bility to subsequent infection with the virus Even before the causative agent of the disease was discovered the nonoccurence of reinfections was so well known that persons who had previously had the disease and re covered volunteered as attendants for the stricken in times of epidemics Reinfections in man have been reported on rare occasions but none of these has been confirmed by immunologic methods or by virus isolation in view of which the accuracy of the reports is open to question It is safe to say that if reinfections take place at all in man they are extremely rare Even in the most susceptible animals such as rhesus monkeys the im munity resulting from infection is effective against massive doses of virus

IMMUNITY FOLLOWING NATURAL OR EXPERIMENTAL INFECTION

In yellow fever in man protective or neutralizing antibody is first detect able on the 4th or 5th day after the onset of illness and may be demon strable before the virus has disappeared from the blood as shown by Berry and Kitchen (1931) The production of antibody continues for some days and the peak level may not be reached until the 4th week or even later Sawyer (1931b) found evidence that over a period of years there may be a gradual decline in the level of protective antibody in the serum following natural infections in man but pointed out that it does not follow that such cases the person becomes again infectible The immunity is life-

long and protective antibody has been demonstrated many years after an attack of the disease Bauer and Hudson (1930) demonstrated its presence in the sera of three persons 23 to 26 years after they had had the disease Sawyer (1931*b*) found the sera of five of six persons protective 75 years after attacks and the serum of another protective 78 years after the attack and he concluded that The persistence of immunity acquired through an attack of yellow fever is not dependent on any subsequent exposure

In the highly susceptible rhesus monkey protective or neutralizing antibody may appear within 4 days after experimental inoculation and thus may be present before the disappearance of virus from the blood (for which the increasing concentration of antibody is doubtless responsible) In these animals antibody is commonly present just before death and may already have brought about the disappearance of the virus from the blood however the virus may even then be recoverable from the liver despite the presence of antibody in the blood In rhesus monkeys which have recovered from active infection the disappearance of antibody has never been reported its persistence for several years has been observed by various workers

The concurrent presence of antibody and virus may greatly increase the difficulty of recovering virus from the blood especially if the subinoculations be made into the homologous species in which as indicated by the work of Bauer (1931*b*) prompt inactivation of the serum proteins does not occur For example Smithburn Haddow and Lumsden (1949) subinoculated normal rhesus monkeys and mice with blood and/or suspensions of liver from moribund or deceased rhesus monkeys that had been infected naturally by being exposed in the forest to the bites of naturally infected mosquitoes In several such instances nonfatal or even inapparent infection occurred in the subinoculated monkeys doubtless owing to the presence of homologous immune globulin in the subinoculated virus-containing materials In all these instances however the subinoculated mice succumbed and virus having the characteristics of pantropic yellow fever virus was recovered from them The differing result in mice was probably due either to inadequacy of the subinoculated antibody when the virus was introduced into direct contact with susceptible cells or to prompt inactivation of the heterologous immune globulin with resultant unmasking of the virus

Other primates experimentally infected with yellow fever virus likewise show early formation of neutralizing antibody Its presence before the virus had disappeared from the blood was noted in several species of African monkeys by Smithburn and Haddow (1949) The occurrence and persist

ence of protective antibody in the serum of wild primates conv from natural infections has proved a valuable tool in epidemiolo vestigations in South America and Africa (Soper 1935b Findlay St poulo et al 1936 Findlay and MacCallum 1937c Burke 1937 Bo Boshell Manrique et al 1944 Laemmert de Castro Ferreira and Ta 1946 Haddow Smithburn et al 1947)

The reason for the persistence of immunity in persons recovered fr naturally acquired yellow fever or in animals recovered from experimen or natural infection is not known Several investigators have attempted t recovery of virus from a variety of tissues with a view to finding an explan tion of the phenomenon in the persistence of virus in the tissues of the im mune animal Penna and Bittencourt (1943) recovered yellow fever virus from the brains of three monkeys that died with generalized tuberculosis 63 93 and 159 days after intracerebral inoculation with the 17D attenuated yellow fever virus The incubation periods in the subinoculated mice were long —9 to 13 days—indicating that the virus recovered was the same as that which had been inoculated and that the quantity of virus present was probably not great in any of the animals These authors failed to recover virus by the same and other methods from seven animals sacrificed 100 to 170 days after similar inoculations The results suggested that tuberculosis infection may play some role in unmasking a latent virus infection Other investigators have met with no success in similar endeavors but as Rivers (1948) has pointed out the failure to recover virus in such cases is not proof of its absence He says

> These agents are intracellularly located and as long as they remain so situ ated are in no danger of being eliminated from the body If they do not kill host cells they can multiply and pass into daughter host cells whenever cellu lar division takes place without coming in contact with or being subjected to the activities of humoral antibodies In this manner it is possible for them to remain indefinitely in an immune host and such is undoubtedly what happens in a number of viral diseases e g virus tumors of chickens and rabbits

Rivers also lists several other virus diseases in which the causative agent been recovered from the immune host Thus although conclusive proof cking the continued residence of the virus in the immune host seems at present time to be the most likely explanation of the enduring immu However there is no evidence to suggest that virus which continues to

reside in an immune host can be transmitted. The intracellular situation
the virus and the presence of specific antibody in the tissues preclude
possibility that the immune host may serve as a source of infection for vec
insects.

The aforementioned responses to infection with yellow fever virus a
typical of those that occur in highly susceptible hosts. The amount of ant
body formed may be related to the extent of multiplication of virus in ther
tissues. By contrast, when a completely resistant host is inoculated with a
small dose of virus or exposed to the bites of infected vector insects, no mul
tiplication of virus can be demonstrated and little or no antibody is pro
duced. A type of response that appears to be intermediate between these ex
tremes was observed by Anderson and Roca García (1947) in experiments on
woolly opossums (*Caluromys laniger*), and it is possible that these and other
animals behave in like manner in cases of natural infection. A considerable
number of the opossums, all of which were nonimmune at the start of the
experiments, were inoculated with small or moderate doses of pantropic
virus. More than half of them exhibited circulating virus, often in small
quantity, and nearly all of those that showed circulating virus developed
neutralizing antibody. The titers of antibody were in many instances low
and a significant number of the animals lost their antibodies within a year.
Reinoculation of such animals sometimes resulted in the reappearance of
the virus in the circulating blood, indicating that the loss of antibody was
correlated with a return of susceptibility. This constitutes unusual behavior,
yet similar results have recently been obtained with other viruses. It seems
likely that such a sequence of events may occur in relatively insusceptible
animals in which the virus does not undergo extensive propagation and
therefore does not evoke a strong antibody response. The phenomenon
could have epidemiologic significance in the event that the degree of viremia
were such as to enable vector insects to become infected.

IMMUNITY FOLLOWING VACCINATION

Three effective and relatively safe methods of vaccination against yellow
fever have been developed:

1. The inoculation of virus modified by serial intracerebral passage in mice
(Sawyer, Kitchen, and Lloyd, 1931 and 1932) or by serial passage in tissue
culture (Lloyd, 1935) together with specific immune serum in quantity suffi
cient to prevent severe reactions.

2 The inoculation of virus attenuated during serial cultivation in vitro ... *Yell*
Theiler and Ricci 1936 Theiler and Smith 1937*a*) with resultan
hepatotropism and diminished residual neurotropic potency i e the
cine (Theiler and Smith 1937*b* Smith Penna and Paoliello 1938)
3 The Dakar method developed by Peltier and his colleagues at the Paste
stitute in Dakar French West Africa (Peltier 1947) This consists in the
application of neurotropic yellow fever virus alone or in combination
vaccinia virus together with gentle scarification of the skin as practice
smallpox vaccination to permit access of the virus or viruses to the tissue
the host

The relative merits and disadvantages of these methods will be discuss
later in this chapter only the effects of vaccination in the production of i
munity in the individual will be considered at this point

Sawyer Kitchen and Lloyd (1932) observed the formation of neutralizing
antibody with regularity in both human beings and monkeys inoculated
with mouse adapted virus together with immune serum The earliest tests
in which protection was obtained in either species were those made on the
7th day following inoculation the latest tests made 20 months after inocula
tion showed that the immunity was retained

The widespread use of the 17D vaccine in South America and during the
recent war throughout the rest of the world has stimulated comprehensive
studies of the immunity evoked by it Theiler and Smith (1937*b*) found pro
tective antibody in the sera of human beings and monkeys 14 days after
inoculation of this virus as vaccine but they were also able to show that
vaccinated monkeys were solidly immune to virulent challenge inoculation
before antibody was demonstrable in their sera Smithburn and Mahaffy
(1945) made a similar study employing a more sensitive method (Smithburn
1945) for performing the protection tests they were able to demonst
antibody in nine of 10 human volunteers by the 10th day and in the
monkeys by the 6th or 7th day Vaccinated monkeys were immune to ch
lenge inoculation from the 5th day onward even though they had not
developed demonstrable antibody

A number of other investigations have been made of the efficacy of th
17D vaccine and of the duration of the immunity that it induces in ma
Soper and Smith 1938*b* Fox and Cabral 1943 Bugher and Gast Galvis
944 Anderson and Gast Galvis 1947 Fox Fonseca da Cunha and Kosso-
udzki 1948 Dick and Smithburn 1949)

From their studies on the results of vaccination Fox and Cabral (1943)

concluded that very young children did not respond as well to 17D vaccin as older persons and that the immunity induced in children endured les well than that induced in adults If true this might necessitate different health and quarantine regulations for persons of different ages However the conclusion was contrary to some of the data that the authors themselves published and was not supported by the findings of Smithburn and Mahaffy (1945) Anderson and Gast Galvis (1947) or Dick and Smithburn (1949) Later Fox Fonseca da Cunha and Kossobudzki (1948) again observed poorer results with sera of children than with those of adults but found that the differences were not statistically significant moreover when they retested by a sensitive method the sera that they had first regarded as nonprotective they found that 42 of 46 actually contained neutralizing antibody A gradual decline in the level of antibody with the passage of years following vaccination is to be expected in that a similar diminution occurs after an attack of yellow fever (Sawyer 1931b) this fact together with the results of the aforementioned studies points to the necessity of employing sensitive methods in studies on the duration of the immunity induced by vaccination It is now known that the protective antibodies induced by the virus usually persist for at least 6 years (Anderson and Gast Galvis 1947 Dick and Smithburn 1949) and it seems not unlikely that the immunity is as enduring as that evoked by an attack of the disease Although the antibody in the blood may never reach the high level induced by an attack of the disease and furthermore may gradually diminish from the peak value (Fox Fonseca da Cunha and Kossobudzki 1948) it does not follow that vaccinated persons again become susceptible field experience indicates that they do not (Bugher Boshell Manrique et al 1944)

PROTECTION OR VIRUS NEUTRALIZATION TESTS

TESTS IN MONKEYS

Stokes Bauer and Hudson (1928b) reported that the highly suscepti e rhesus monkey could be protected against inoculation with virulent low fever virus by the simultaneous injection of serum from a human ng recovered from the disease Until mice were found to be susceptible he virus the only method available for detecting yellow fever antibody the protection or virus neutralization test in monkeys The technique

2 The inoculation of virus attenuated during serial cultivation in vitro (Lloyd Theiler and Ricci 1936 Theiler and Smith 1937a) with resultant loss of hepatotropism and diminished residual neurotropic potency i e the 17D vaccine (Theiler and Smith 1937b Smith Penna and Paoliello 1938)

3 The Dakar method developed by Peltier and his colleagues at the Pasteur Institute in Dakar French West Africa (Peltier 1947) This consists in the topical application of neurotropic yellow fever virus alone or in combination with vaccinia virus together with gentle scarification of the skin as practiced in smallpox vaccination to permit access of the virus or viruses to the tissues of the host

The relative merits and disadvantages of these methods will be discussed later in this chapter only the effects of vaccination in the production of immunity in the individual will be considered at this point

Sawyer Kitchen and Lloyd (1932) observed the formation of neutralizing antibody with regularity in both human beings and monkeys inoculated with mouse adapted virus together with immune serum The earliest tests in which protection was obtained in either species were those made on the 7th day following inoculation the latest tests made 20 months after inoculation showed that the immunity was retained

The widespread use of the 17D vaccine in South America and during the recent war throughout the rest of the world has stimulated comprehensive studies of the immunity evoked by it Theiler and Smith (1937b) found protective antibody in the sera of human beings and monkeys 14 days after inoculation of this virus as vaccine but they were also able to show that vaccinated monkeys were solidly immune to virulent challenge inoculation before antibody was demonstrable in their sera Smithburn and Mahaffy (1945) made a similar study employing a more sensitive method (Smithburn 1945) for performing the protection tests they were able to demonstrate antibody in nine of 10 human volunteers by the 10th day and in rhesus monkeys by the 6th or 7th day Vaccinated monkeys were immune to challenge inoculation from the 5th day onward even though they had not yet developed demonstrable antibody

A number of other investigations have been made of the efficacy of the 17D vaccine and of the duration of the immunity that it induces in man (Soper and Smith 1938b Fox and Cabral 1943 Bugher and Gast Galvis 1944 Anderson and Gast Galvis 1947 Fox Fonseca da Cunha and Kossobudzki 1948 Dick and Smithburn 1949)

From their studies on the results of vaccination Fox and Cabral (1943)

concluded that very young children did not respond as well to I
as older persons and that the immunity induced in children end
well than that induced in adults. If true, this might necessitate
health and quarantine regulations for persons of different ages. H
the conclusion was contrary to some of the data that the authors the
published and was not supported by the findings of Smithburn and M
(1945), Anderson and Gast-Galvis (1947), or Dick and Smithburn (1
Later Fox, Fonseca da Cunha, and Kossobudzki (1948) again obse
poorer results with sera of children than with those of adults but found t
the differences were not statistically significant; moreover, when they retest
by a sensitive method the sera that they had first regarded as nonprotectiv
they found that 12 of 16 actually contained neutralizing antibody. A gradu
decline in the level of antibody with the passage of years following vacci-
nation is to be expected in that a similar diminution occurs after an attack
of yellow fever (Sawyer, 1931*b*); this fact, together with the results of the
afore-mentioned studies, points to the necessity of employing sensitive
methods in studies on the duration of the immunity induced by vaccination.
It is now known that the protective antibodies induced by the virus usually
persist for at least 6 years (Anderson and Gast-Galvis, 1947; Dick and Smith-
burn, 1949), and it seems not unlikely that the immunity is as enduring as
that evoked by an attack of the disease. Although the antibody in the blood
may never reach the high level induced by an attack of the disease and
furthermore may gradually diminish from the peak value (Fox, Fonseca da
Cunha, and Kossobudzki, 1948), it does not follow that vaccinated persons
again become susceptible. Field experience indicates that they do not
(Bugher, Boshell-Manrique, et al., 1944).

PROTECTION OR VIRUS-NEUTRALIZATION TESTS

TESTS IN MONKEYS

Stokes, Bauer, and Hudson (1928*b*) reported that the highly suscepti-
ble rhesus monkey could be protected against inoculation with virulent
ellow fever virus by the simultaneous injection of serum from a human
ing recovered from the disease. Until mice were found to be susceptible
the virus, the only method available for detecting yellow fever antibod
the protection or virus-neutralization test in monkeys. Th

as originally employed consisted in the subcutaneous or intraperitoneal inoculation of 0 1 to 1 0 cc of convalescent serum and at the same time 1 0 to 1 5 cc of serum from an infected monkey Control animals received nonimmune serum and the same amount of virus containing monkey blood The latter almost invariably succumbed while those receiving human convalescent serum survived and were subsequently refractory to challenge reinoculation Bauer (1931*b*) showed that the immune serum of rhesus monkeys (homologous species) may be effective when given as long as 3 to 6 weeks prior to the inoculation but that the serum of a heterologous species (man) is without protective effect if given more than 1 week before the virus N C Davis (1934*b*) showed that immune serum has some protective action up to 48 hours after inoculation but none if delayed until the onset of fever The original method or slight modification thereof was employed by Aragão (1928) N C Davis (1929) Hudson Bauer and Philip (1929) Hudson Philip and Davis (1929) and Sawyer Kitchen et al (1930) to show that the yellow fever of Africa and that of the Americas are the same and by the last mentioned investigators to show that leptospiral infection is unrelated to yellow fever Bauer and Mahaffy (1930*a*) utilized it to show that African monkeys infected experimentally with yellow fever virus develop protective antibody in consequence The method was also employed for retrospective diagnosis (Hudson and Kitchen 1930 N C Davis 1931*d*) for studies of the duration of immunity in man (Bauer and Hudson 1930 Sawyer 1931*b*) and for the first surveys directed toward determining the geographic distribution of the disease (Beeuwkes Bauer and Mahaffy 1930 Soper Frobisher et al 1932) Although the results obtained with it were quite satisfactory the use of the method for immunity surveys was so expensive that efforts were soon directed toward finding a less costly technique for this purpose

TESTS IN GUINEA PIGS

Sellards (1930) attempted the serial passage of yellow fever virus in guinea pigs by extraneural inoculation with success in two of three trials Later Sawyer and Frobisher (1932) conducted similar experiments likewise with partial success The virus did not cause death in guinea pigs but it could sometimes be transmitted back to rhesus monkeys by subinoculation of blood The latter authors were able to infect guinea pigs by the bite of experimentally infected specimens of *Aedes aegypti* and further to transmit the disease from guinea pig to rhesus monkey either by the bites of aegypti

or by subinoculation of blood It was obvious from these experiments however that the extraneural inoculation of guinea pigs with yellow fever virus did not induce an infection that was utilizable for experimental purposes

Theiler (1933*b*) after his discovery of the susceptibility of Swiss mice to the intracerebral inoculation of yellow fever virus showed that guinea pigs are susceptible to intracerebral inoculation of mouse adapted virus and Lloyd Penna and Mahaffy (1933) likewise were successful in establishing the virus in guinea pigs and in devising an intracerebral protection test in these animals It was found however that the virus propagates less well in the brains of guinea pigs than in the brains of mice and that protection tests employing guinea pigs are not only more expensive but also less satisfactory than those in mice

INTRACEREBRAL PROTECTION TEST IN MICE

Theiler (1931) demonstrated the protective action of specific immune serum against yellow fever virus when the two are mixed and inoculated intracerebrally into susceptible white mice The length of contact between the serum and virus prior to inoculation seemed to be of little importance Later he (Theiler 1933*a*) extended his studies and developed the following method Mice of high and uniform susceptibility to the virus are used The virus is a desiccated preparation of infected mouse brain the potency of which is repeatedly assayed so that the dosage may be carefully gauged Equal parts of test or control sera and of a dilution of the virus calculated to deliver 100 LD_{50} when combined with the serum are mixed and inoculated intracerebrally in 0 03 cc quantities per mouse

All or nearly all mice receiving sera containing the specific antibody are protected whereas mice receiving virus mixed with nonimmune serum succumb to yellow fever encephalitis on the 4th to the 10th day both inclusive Theiler emphasized the importance of a suitable protein-containing diluent and for accurate work a suitably large number of mice Bugher (1940) in applying the method to a study of sera from wild animals and human beings introduced a uniform period of incubation of serum virus mixtures prior to inoculation and demonstrated the necessity for this procedure He subjected his results to statistical analysis and showed that if the accepted criteria of survival ratios alone were used in interpreting the test results a significant proportion of weakly protective sera would be regarded as nonprotective By careful study of the variability of results with sera

from nonimmune individuals of a given species he found that the average survival time is a more sensitive key to the detection of weak positives than is the survival ratio He observed considerable variation in the average survival time with sera of different species of animals and found that with some it was necessary to give larger than usual doses of virus in order to reduce the variation in survival time

The intracerebral mouse protection test for antibody to yellow fever virus has been widely used in recent years in particular in the yellow fever investigations in Colombia and Brazil The method requires careful attention to a number of technical details (Bugher 1940) and deserves critical interpretation of data When these precautions are observed the results are excellent The principal obstacle to the general use of the method is that in some tropical countries where the tests are performed the materials necessary for the preparation of large quantities of desiccated virus cannot readily be obtained

INTRAPERITONEAL PROTECTION TEST IN MICE

Sawyer (1931a b and c) and Sawyer and Lloyd (1931) devised a method for the performance of yellow fever protection tests in mice wherein the mixtures of serum and neuroadapted virus are inoculated intraperitoneally Ordinarily the virus has little effect when injected by this route but the method includes a preliminary intracerebral inoculation of sterile 2 per cent starch solution to produce a mild trauma in the brain which permits localization of virus there from the blood With this method deaths occurring within 4 days following inoculation are regarded as nonspecific The intraperitoneal route of inoculation of the serum virus mixtures permits the administration of a larger volume of serum and obviates the necessity for the previous meticulous assay of the virus which the intracerebral test requires Mouse brain passage virus may be used whereas the intracerebral test is best done with highly standardized desiccated virus Minor modifications of the original methods have been adopted in various laboratories in part to meet local conditions and in part to increase the sensitivity of the test thus permitting the detection of immune individuals having low levels of antibody However the intraperitoneal test essentially as originally conceived has been used more than any other and was the principal method employed in most of the great yellow fever immunity surveys that covered the larger part of the world

The criteria adopted by Sawyer and Lloyd (1931) for the interpretation of results based on mortality have proved suitable not only for their own technique but also for all the modifications thereof for the intracerebral test and for interpretation of similar tests with other viruses The scheme is therefore reproduced herewith altered only to show clearly the range of reactions that must be regarded as inconclusive (Table I)

TABLE 1

INTERPRETATION OF RESULTS OF PROTECTION TESTS

Number of mice living on the 4th day	*Number of mice living on the 10th day*		
	Negative	*Inconclusive*	*Positive*
4	0	1 to 3	4
5	0 1	2 3	4 5
6	0 1	2–4	5 6
7	0–2	3 4	5
8	0–2	3 5	6–8
9	0–2	3–6	9
10	0–3	4–6	10
11	0–3	4–	8 11
12	0–3	4–8	9–12

Source Based on Sawyer and Lloyd 1931

The time of death of mice in yellow fever protection tests is influenced by various factors including susceptibility of the mice route by which they are inoculated the concentration and passage level of the virus and perhaps climatic or other local conditions In some instances (intracerebral test in adult mice or intraperitoneal test in infant mice) it has proved desirable or necessary to accept 3 instead of 4 days as the period subsequent to which all deaths are regarded as specific The usual period of observation of mice is 10 days but in tests employing very young mice observations should be continued for an additional 2 to 4 days Aside from this the foregoing criteria for interpretation of mortality results are not likely to require modification

Experience of several workers has shown that some species or individuals respond to infection with yellow fever virus by producing antibodies in rather low titer It has also been found that the levels of antibody evoked by vaccination with the 17D virus are as a rule not so high as those follow

ing natural infection (in man) with unmodified virus For surveys of immunity in wild animals or in human beings who have been vaccinated special methods of interpretation or special techniques for testing sera may be required Bugher (1940) using the intracerebral mouse protection test found that sera from some animal species must be tested against larger doses of virus than is necessary with sera from man or from rhesus monkeys in order to establish a workable nonimmune range He showed however that the mortality results alone may be misleading and that the average survival time is a more reliable guide to a decision as to whether or not a given serum contains antibody

Whitman (1943) found that mice 18 to 21 days of age are sufficiently more susceptible than adults to justify carrying out intraperitoneal tests on them without preliminary intracerebral inoculation of starch solution His method permits the performance of tests with as little as 0 4 cc of serum whereas the standard test in adult mice requires 3 0 cc Further he observed that the intraperitoneal test in 18 to 21 day old mice is more sensitive and therefore of enhanced usefulness in testing sera containing small amounts of antibody During the course of these investigations it was found that

1 The susceptibility of mice to yellow fever virus inoculated extraneurally is highest in early infancy and declines progressively at least to the 30th day of life
2 The decreasing susceptibility is primarily a function of age but is also influenced independently by rate of growth
3 There are no significant sex linked differences in susceptibility in 21 day old mice inoculated extraneurally
4 Even though all may die the prolonged average survival time of groups of mice in the standard intraperitoneal test of Sawyer and Lloyd is usually associated with the presence of antibody that can readily be demonstrated in a more sensitively balanced test

Experimental studies on the yellow fever protection test carried out in Uganda (Smithburn 1945) confirmed the observation that enhanced sensitivity may be obtained in the intraperitoneal protection test by using mice of such early age and in consequence of such high susceptibility that preparatory intracerebral injection of an irritant is unnecessary In these studies it was clearly shown that sera that are low in antibody may be protective by one test method and nonprotective by another depending on the sensitivity of the test Higher virus titers were obtained in very young mice by

intraperitoneal inoculation than in adult mice by intracerebral inoculation but it was found that the antibody is more effective when the serum virus mixtures are given intraperitoneally to immature mice than when adult mice are used as was previously observed by Olitsky and Harford (1938) with equine encephalomyelitis virus A pool of yellow fever immune serum gave an antibody titer of 1 in 59 against 16 LD_{50} of virus in an intracerebral test in adult mice and a titer of 1 in 128 against 18 LD_{50} of virus in an intra peritoneal test in mice of the same age that had had a preliminary intracere bral inoculation of sterile starch solution Even more striking was the result in 14-day old mice an antibody titer of 1 in 256 against 10 times as much virus (200 LD_{50})

The quantities of virus referred to here are the effective units for mice of the stated age by the technique specified as determined in titrations by each technique of virus mixed with nonimmune serum and tested in the same doses as in the case of the immune serum

The result of these studies was the adoption for use in East Africa of an intraperitoneal protection test employing 1 per cent mouse brain passage virus in a 10 per cent serum saline diluent mixed with serum in a propor tion of 1 to 2 and inoculated in 0 06 cc quantities into eight or ten 14-day old (or slightly younger) mice or in 0 6 cc quantities into groups of six starch treated mice 35 to 42 days old The choice between infant or adult mice for this method was made in accordance with the amount of serum on hand and the availability of infant mice of appropriate age Controls included a titration of the virus in nonimmune serum and a titration of a known immune serum against the test 1 per cent virus This method was used with notably improved results from 1942 onward not only in human immunity surveys (Smithburn Goodner et al 1949) but also in postvacci nation surveys (Smithburn and Mahaffy 1945 Dick and Smithburn 1949) and in surveys of immunity in wild animals (Haddow Smithburn et al 1947)

SUBCUTANEOUS PROTECTION TEST IN INFANT MICE

Kerr and Bugher have made comprehensive experiments with the yellow fever protection test employing 3 to 5 day-old mice and the subcutaneous route for the inoculation of serum virus mixtures Mice of this age are approximately as susceptible to subcutaneous inoculation as are adult mice to intracerebral inoculation (Bugher 1941) They therefore do not require

in ... starch solution as do adult mice that are to
n ... ently Nevertheless specific antibody appears to
... infant mice inoculated subcutaneously than in
... acerebrally The method has been found applicable
... the protection test but has not been adopted for
... reason that equally effective methods were already

... WITH STRAINS OTHER THAN THE NEUROTROPIC VARIANT

... methods and techniques for performing the yellow ... in mice have employed Theiler's neuroadapted strain ... it is necessary or desirable to carry out yellow fever ... with other strains For example when a strain of virus is ... the custom now is to identify it by testing it against one or ... known to contain specific antibody against yellow fever virus ... in Mice (Smithburn Haddow and Lumsden 1949) showed ... intraperitoneal test is often inadequate for this purpose as the ... of freshly isolated pantropic virus by intraperitoneal inoculation ... even after preparation of the mice by intracerebral injection ... starch solution that massive doses fail to cause death In such ... intracerebral test serves the need but must be done by a special ...

According to this method several dilutions of mouse brain virus or monkey ... virus are mixed with equal amounts of normal serum and yellow ... immune serum the preparations ... then incubated ... ulated ... into separate groups ... 12 mice Mul... tions ... virus-containing materials are ... owing to ... at the ... quantitative content of virus is unk... also bec... of ... may invalidate the test In such ... e yellow ... une serum shows signif... ation ... is ... the agent in question is ... er virus

It may also be desi... form yell... where yellow fever ... red a... unwise to import any ... al o... For example in rec... undertaken at the Sc...

Johannesburg Yellow fever has never been shown to have occurred there, and a method was sought for performing protection tests without the importation of a virus which might have, or might acquire, hepatotropic affinities The 17D yellow fever vaccine virus, which cannot be transmitted by mosquitoes, was therefore used in an intracerebral protection test, according to methods previously employed with the neuroadapted virus, and satisfactory results were obtained The use of this virus, which has an incubation period of 7 to 12 days, depending on the dose, requires that the mice be observed for 21 days Otherwise, the method and the interpretation of results are similar to those for the intracerebral test with neuroadapted virus (Gear, 1945)

IMMUNOLOGIC RELATIONSHIP OF STRAINS OF YELLOW FEVER VIRUS

Although there are marked differences in the pathogenic properties of different strains of yellow fever virus and although there are some strains that may be more strongly antigenic than others, these facts do not indicate any variability in the antigenic make up of the strains Furthermore, the modification of virus by neuroadaptation or by such laboratory procedures as in vitro cultivation apparently does not induce changes in the qualitative antigenic composition of the agent All strains of the virus, whatever their source or history, have proved to be reciprocally cross reactive in neutralization tests The fact that most recently isolated strains induce the formation of complement fixing antibody whereas the attenuated 17D strain does not, is no indication of antigenic variability owing to the fact that the complement fixing antibody apparently is not evoked by the virus per se In this respect the immunology of yellow fever is much simpler than that of certain other virus diseases such as influenza in which there is great variability in the antigenic composition of different strains of the causative agent

THE NATURE OF THE VIRUS–ANTIBODY REACTION

The effects of the specific antibody upon yellow fever virus have not been determined, beyond demonstrating that the pathogenic properties of the virus may be rendered ineffectual by the reaction Whether an appropriate amount of antibody will kill the virus or make it irreversibly incapable of

propagation or whether the virus remains viable and potentially capable of regaining all or part of its pathogenic properties is not known Some investigations of these questions have been made and certain of the observations are worthy of mention

Theiler made mixtures of potent virus preparations with yellow fever immune serum in such proportions that the mixtures were neutral i e nonpathogenic when inoculated intracerebrally He then made dilutions of the mixtures and tested these to ascertain whether active virus could thus be liberated No active virus was demonstrated by this method indicating perhaps that the virus had been killed or irreversibly inactivated However the relative amounts of serum and virus that are brought into contact may influence the resulting reaction as shown by the following experiences

Bugher and Smith (1944) cultivated the 17D strain of virus in vitro in chick embryo–Tyrode s solution medium to which they added human yellow fever immune serum The same pool of immune serum was used throughout their work Preliminary tests showed that the addition of amounts of this serum in excess of 0 2 per cent resulted in the disappearance of the virus by the 3d subculture With smaller amounts than 0 2 per cent the virus propagated well and it was passed through 57 serial subcultures each of which contained 0 1 per cent of the immune serum Parallel cultures were made in medium which was identical in all respects except that the serum added was devoid of demonstrable antibodies From the 57th subcultures in each series lots of vaccine were prepared by identical methods The two vaccines proved to have approximately equal amounts of virus and to be equally antigenic Thus it is clear that a subneutralizing quantity of antibody does not prevent multiplication of virus or render the propagating virus antigenically ineffectual

Experience with the protection test in rhesus monkeys also yielded some information on the effect of antibody on the virus The administration of a suitable quantity of immune serum to a monkey will suffice to prevent the lethal effects of the virus yet the monkey acquires an active immunity as a result of the experience Thus the abolition of pathogenic properties does not necessarily eliminate the antigenic properties of the virus

No conclusive evidence is available to indicate whether agglutination or lysis occurs as a result of the contact between specific antibody and yellow fever virus There is however some indirect evidence that may indicate

that virus particles are agglutinated by antibody If relatively concentrated yellow fever virus say 1 per cent mouse brain be mixed with an equal quantity of specific immune serum and inoculated intraperitoneally into 14 day-old mice few if any of them will succumb If the same mixture be inoculated intracerebrally into mice of any age there is a strong probability that all of them will die Thus the serum virus mixture may be neutral by intraperitoneal but not by intracerebral inoculation It is possible that this occurs because of the fact that the antibody agglutinates the virus and prevents its egress from the peritoneal cavity in the one instance whereas in the other the virus particles are introduced directly into contact with susceptible cells and as there is insufficient antibody to mask all the virus death ensues

Bugher conducted an exploratory experiment to determine whether the virus particles are aggregated by specific antibody He found that when the virus was mixed with antibody it did not pass through graded collodion membranes which normally permit its passage Although unequivocal proof that agglutination occurs is lacking this nevertheless seems a possible and a reasonable explanation of the phenomenon Direct evidence of the nature of the reaction may be obtainable by present day physical methods

VARIABLES IN MOUSE PROTECTION TESTS

Theoretically it should be possible to perform protection tests in such ashion that the inoculation invariably results in death if antibody is absent nd in survival if antibody is present However so many factors contribute the variability of results that this ideal cannot be achieved with regularity large series of tests

Mice of different strains vary in their susceptibility to the virus (Sawyer Lloyd 1931) and it has been the experience of several workers that nall proportion of mice within a given highly susceptible strain may esistant With a given strain of mice the susceptibility by extraneural lation is highest in early infancy (Theiler 1930b) and declines pro ely with increasing age (Whitman 1943) so that if immature mice be used their age and their susceptibility at the age of use must be ely known

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nique mouse passage virus may be satisfactorily employed but it is possible that with this method also best results might be obtained with desiccated virus of known potency Whatever technique is employed it is essential that all dilutions of virus be prepared in a diluent that will hold to a minimum the deterioration of the virus within the time required for performance of the test It will be recalled that saline solutions are deleterious to yellow fever virus (Bauer and Mahaffy 1930*b*) if they are used as diluents without the addition of some protein that prevents the destructive effects No diluent has yet been found that is superior to 10 per cent nonimmune monkey serum in physiologic saline

Minor degrees of hemolysis of the serum to be tested do not influence results appreciably However extreme hemolysis and autolysis of clots do adversely affect the results and are to be avoided Bacterial contamination of serum may give rise either to false positive or to false negative results (apparent if the contaminant is a pathogen or actual if the antibody has been destroyed by proteolytic enzymes) Filtration of contaminated specimens may be resorted to but the results would even then be subject to question Yellow fever antibody is quite stable (Bugher 1945) and the refrigeration of serum samples is not essential except possibly in cases where storage is necessary for long periods prior to testing

A minimum of six mice should be used for each test or control specimen regardless of the technique employed For the testing of sera from vaccinated persons or others in whom the level of antibody may be low larger numbers of mice give more reliable results (Theiler 1933*a* Whitman 1943) This is especially true in the testing of sera from wild animals for immunity survey purposes (Bugher 1940)

Time factors are of importance in the performance of protection tests if the best results are to be obtained Sera should be prepared and appropriately labeled in advance and all the materials required for the test including mice should be at hand before the preparation of the virus is undertaken Once the preparation of virus is started the entire test should be carried out as expeditiously as meticul[illegible] k permits in [illegible] minimize the deterioration of virus Each [illegible] test should [illegible] zed as to time so that the serum virus [illegible] is as ne[illegible] t as possible

With the intraperit[illegible] in [illegible] dult mice [illegible] r val between the intra[illegible] h sol[illegible]

peritoneal inoculation of virus is of the utmost importance The followi experiment performed by Goodner and Smithburn at the Yellow Fev Research Institute Entebbe Uganda illustrates this point

Serial fourfold dilutions of freshly prepared mouse passage French neuro tropic yellow fever virus varying from 1 in 100 to 1 in 25 600 suspended in 10 per cent nonimmune human serum saline were added to whole non immune human serum in proportion of 1 part to 2 and intraperitoneal titra tions were done in four series of susceptible Swiss mice 34 to 36 days old One series of mice had received intracerebral starch inoculations 3 hours previously a second series had received the starch 1 hour previously a third series received it immediately preceding the intraperitoneal inocula tion of serum and virus and the fourth series received it 30 minutes after the serum virus inoculations A second part of the same experiment consisted in the intraperitoneal titration of a known immune human serum pool against 1 in 100 virus at the same time intervals with reference to the intra cerebral inoculation of starch One volume of the 1 in 100 virus was added to two volumes of serum dilutions and the inoculations were made at the above stated intervals in relation to starch inoculations into Swiss mice 34 to 36 days of age The experiment was so planned and executed that the last inoculations of serum virus mixtures were finished within 81 minutes of the beginning of preparation of the virus suspension thus there was little opportunity for deterioration of the virus All intracerebral inocu lations were done by one person and the intraperitoneal inoculations by another both of whom were highly skilled and experienced technicians Deaths occurring in the first 3 days were excluded from consideration as being nonspecific Mice dying between the 4th and 10th days and any that were moribund or paralyzed on the 10th day were regarded as being specifically infected All others were regarded as survivors End points were determined by the method of Reed and Muench (1938) and the end point dilution for virus was regarded as containing one infectious unit per volume inoculated Results of the experiment are shown in Table 2

These results indicate the great variation to be expected if the intervals between starch injection and virus inoculation are not rigidly controlled f the starch injection were administered too far in advance the effective rus potency might be so low as to give a result simulating protection with ra devoid of antibody The starch injection has its maximum effect when en after the intraperitoneal inoculation of serum and virus but with this

procedure a potent immune serum might appear to be weak or nonprotective. Although neither the virus nor the antibody titer is greatest when the two inoculations are made one immediately after the other, the results are subject to less variation and the intervals are more amenable than any other to control. It is therefore recommended that the inoculations be given in this manner, as originally proposed by Sawyer and Lloyd (1931).

TABLE 2

EFFECT OF THE TIME INTERVAL BETWEEN INTRACEREBRAL INOCULATION OF STARCH SOLUTION AND THE INTRAPERITONEAL INOCULATION OF SERUM VIRUS MIXTURE IN PROTECTION TESTS

No. of minutes between starch treatment and inoculation of serum virus	*Titer of virus mixed with nonimmune serum*	*Units effective virus in 1:100 dilution*	*Titer of antibody vs. 1:100 virus*
	1 in		1 in
+180 *	240	2.4	1,024
+ 60 *	794	7.9	568
0	992	9.9	96
− 30 †	8,960	89.6	9

* Starch inoculated prior to serum virus mixtures
† Starch inoculated after serum virus mixtures

The incubation of serum virus mixtures to be employed in the protection test has a definite, although relatively small, effect on results. The total effect is evidently the result of two factors: (a) gradual diminution of the potency of the virus suspension with the passage of time, and (b) interaction of antibody and virus. The first factor tends to be limited by the use of a suitable protein-containing diluent and is of course less with shorter periods of incubation. Bugher studied the effect of incubating serum virus mixtures for the intraperitoneal protection test. He made intraperitoneal inoculations into starch-treated adult mice immediately after the preparation of serum virus mixtures, then repeated the inoculations with serum virus that had been incubated for 2 hours at 37°C. With a 2 hour period of incubation he obtained a decrease of intraperitoneal virus potency from 1 in 71 to 1 in 66, but the antibody potency showed an increase in the same interval of from 1 in 37 to 1 in 143.

An experiment of similar nature to the foregoing, but employing the intracerebral route of inoculation, was made in East Africa. Serial dilutions

of virus were mixed with two volumes of normal serum and inoculat at once into normal mice Three decimal dilutions of virus those imm diately preceding the expected end point were mixed with two volume of dilutions of a known immune serum and intracerebral inoculations were made at once All the serum virus mixtures were then incubated 1 75 hours at 37°C and the inoculations were repeated All the mice were from the same stock and their ages varied from 44 to 48 days All inoculations were made by the same well trained technician Incubation of a given serum virus mixture was started immediately after the first inoculations were done so that the final inoculations in the experiment were made within 2 hours and 38 minutes after the commencement of the injections Twelve mice were inoculated with each serum virus mixture at each interval and an additional 30 mice per dilution of virus were inoculated with the three critical normal serum virus mixtures The results are given in Table 3 they show that although there was no significant change in the virus titer with incubation there was an increase of three to eightfold in the antibody titer following incubation the magnitude of the increase being greatest with the larger doses of virus It is therefore obvious that the interaction of antibody and virus is not instantaneously completed and that a period of incubation has an enhancing effect on the action of the specific antibody

As a rule all inoculations in a given test run should be done by a single well trained technician but if the intraperitoneal test in starch treated adult mice is employed it is best to have the intracerebral injections of starch solution and the intraperitoneal injections of serum virus mixtures done in immediate succession by separate technicians working side by side The latter procedure minimizes the variability attendant upon differences in the interval between injection of starch and inoculation of serum virus mixtures and furthermore permits both injections to be given with a single induction of anesthesia

The importance of having all inoculations given by one experienced per son is illustrated by the following experiments carried out in Uganda the results of which bear not only upon protection tests but also upon any uantitative work with viruses Three ampules of a good lot of desiccated rench neurotropic yellow fever virus were rehydrated in distilled water oled and used for the preparation of serial decimal dilutions The diluent s 10 per cent nonimmune rhesus monkey serum in physiologic saline ficient quantities of each dilution to be used for inoculation were placed

TABLE 3

SURVIVAL RATIOS AND TITERS OF VIRUS AND ANTIBODY IN MICE INOCULATED WITH FRESHLY PREPARED OR WITH INCUBATED SERUM VIRUS MIXTURES

Virus dilution	*Titration of virus with normal serum*		*Titration of antibody*			
	Fresh	*Incubated*	*Fresh*		*Incubated*	
	Survival ratio	*Survival ratio*	*Survival ratio*	*Titer*	*Survival ratio*	*Titer*
				1 *in*		1 *in*
10^{-4}	0/42 *	0/42	12/71 †	2	27/71	16
10^{-5}	0/42	0/41	25/72	9	39/72	50
10^{-6}	4/40	9/41	50/71	163	60/72	512
10^{-7}	10/12	7/12				
10^{-8}	12/12	11/12				
10^{-9}	12/12	12/12				
Titer	$10^{-8.5}$[illegible]	$10^{-8.38}$				

* *Numerator represents number of mice surviving denominator the number inoculated and alive on the 3d day*

† *Survival ratios of mice in antibody titrations include all mice used in tests against that dilution of virus without regard to dilution of antibody*

in flasks and all inoculations of any one dil…
flask Five pers…
tions each c… …ilution When these inoculations were …pleted they were repeated in the reverse order the last person to inoculate in the 1st series being the first in the 2d series All mice for the tests were from the same breeding stock and those used in each test were within 4 days of the same age The 2d and 3d experiments were done in identical manner except that fresh mouse brain passage virus was used and the original 10 per cent suspensions were Seitz filtered Three of the persons (Nos 1 2 3) who gave inoculations had had long experience in this work but individual No 3 had had little recent practice Individuals Nos 4 and 5 were less experienced The experiments were done to ascertain how closely each of the five persons could duplicate his own results and how much variation would occur from one individual to another Results of the three tests expressed as logarithms of virus titers are shown in Table 4

TABLE 4

RESULTS OF SUCCESSIVE INTRACEREBRAL TITRATIONS OF THE SAME VIRUS SUSPENSIONS IN DUPLICATE BY EACH OF FIVE PERSONS

Individual giving inoculation	*Logarithms of virus titers*					
	Experiment 1		*Experiment 2*		*Experiment 3*	
	Series 1	*Series 2*	*Series 1*	*Series 2*	*Series 1*	*Series 2*
No 1	6 83	6 65	7 60	7 44	7 00	00
No 2	6 8	6 73	7 84	7 79	7 49	44
No 3	5 93	5 7	7 45	7 51	6 67	6 52
No 4	7 07	6 86	6 88	7 77	7 55	6 86
No 5	6 39	6 44	7 0	7 5	6 70	6 86

The foregoing results show that the same virus preparation inoculated by different persons may give very different titers and that even with groups of 12 mice per test there may be quite divergent results following duplicate series of inoculations by the same person On the basis of these results individuals Nos 1 and 2 thenceforth did all the inoculations in any experiments of quantitative nature

Another source of variability in protection test results and perhaps one of the most important is concerned with inherent properties of the serum of different animal species With sera from human beings and most wild primates there is relatively little variability either in mortality rate or mean survival time except as occasioned by the presence or absence of antibody With certain other species of mammals and especially with the sera of cattle sheep and birds results may be variable quite independently of the presence or absence of antibody Findlay Stefanopoulo et al (1936) obtained weakly protective results with the sera of sheep that could not have been immune to yellow fever MacCallum and Findlay (1937) reported the presence of virucidal properties in low titer in the serum of cattle from regions in which yellow fever was not known to be endemic The occurrence of virucidal properties in the sera of these and other domestic animals including horses pigs camels and dogs was noted by Findlay (1941) Saleun (1939) and E C Smith (1940) Work with sera of the animal groups mentioned has not been sufficient to provide an answer to the question of

whether or not any of the animals ever acquire specific immunity to yellow fever but it is highly probable that some of the results with sera of *Bovidae* and *Ovidae* do not represent specific neutralization The factors responsible for the nonspecific virucidal property of serum of these species have not been determined but this limitation of the protection test is now generally accepted Similar nonspecific false positive reactions have been observed with the sera of certain birds (Bugher 1940) In addition the sera of some birds impose technical difficulties that complicate the performance of protection tests in that clotting of the mixture may occur when mouse brain virus is added to the avian serum Preclotting and separation of the liquid component or heat inactivation did not regularly suffice to eliminate this difficulty

In addition to the foregoing difficulties it was found by Bugher (1940) that in testing the sera of certain species of wild animals by methods applicable to the testing of sera of human beings or wild primates there is such a wide spread in the survival times of mice that interpretation of results is unreliable It was found that this could be reduced and that assessable results could be obtained if the dose of virus was increased but the amount of virus required for such species had to be determined by experiment

Koprowski (1946) has reported nonspecific neutralization of the virus of yellow fever and other viruses by the sera of certain Brazilian wild animals notably rodents and marsupials The neutralizing property was found to disappear and reappear on occasion This together with the fact that the sera neutralized viruses (West Nile St Louis or Japanese B) not known to occur in Brazil was the reason for considering the reactions nonspecific Attempts to eliminate such nonspecific neutralizations by the use of larger doses of virus or by heat inactivation of the sera were unsuccessful Laemmert de Castro Ferreira and Taylor (1946) obtained neutralization that was probably nonspecific with sera of *Marmosa* spp but their results with 27 specimens from *Didelphis marsupialis* were consistently negative in sharp contrast to the results of Koprowski

The disappearance and reappearance of virus neutralizing properties (Koprowski 1946) in the serum of a given animal if unrelated to any experience with the virus in question would constitute a serious limitation to the use of the protection test for the animal species in question However such discordant results would seem to point more to the need for closer scrutiny of the methods and the controls used in the tests and to investigation of the sterility of the sera than to a peculiarity of the animal species

concerned Furthermore the fact that neither West Nile St Louis nor Japanese B virus has ever been isolated in Brazil does not constitute adequate evidence that no agent immunologically related to these three is active there In fact Hughes and Perlowagora (1950*a*) have found that the Ilhéus virus isolated in Brazil (Laemmert and Hughes 1947) cross reacts in the complement fixation test with the St Louis virus Neither does it follow that a positive result in a yellow fever protection test with the serum of a wild primate is nonspecific if the sample also neutralizes another virus (Japanese B) as Kumm and Laemmert (1950) have assumed The demonstrated specificity of the yellow fever protection test with sera from primates must be accepted until proof of its nonspecificity is found

SPECIFICITY OF THE PROTECTION TEST WITH SERA FROM HUMAN BEINGS AND WILD PRIMATES

Hughes and Sawyer (1932) reported the results of protection test surveys with the sera of residents of Canada and China in whom the likelihood of any experience with yellow fever virus was slight None of 88 sera in the latter sample was protective but one of 109 Canadian sera gave results variously inconclusive or protective in six of seven tests Sawyer Bauer and Whitman (1937) tested the sera of 876 persons residing in various countries of the Middle East and Far East where yellow fever had never been reported and found only two that were protective Both of these were from residents of Chingleput Madras One was tested repeatedly and exhibited yellow fever virucidal properties to a degree fully comparable with that of serum taken from individuals a few years after attacks of the disease This serum was also tested against the viruses of lymphocytic choriomeningitis and St Louis encephalitis to learn whether the nonspecific power would protect against other virus diseases but no protective properties against these agents were demonstrable Thus it is evident that on rare occasions the serum of a person who has had no known contact with yellow fever virus may give a positive reaction in a protection test The incidence of such results in these studies was 0 28 per cent

Other diseases of viral bacterial or protozoan etiology do not give rise to the formation of neutralizing antibody against yellow fever virus to an extent that is likely to be misleading Theiler found that sera containing antibody against dengue virus moderately increased the survival time of mice in yellow fever protection tests but did not prevent death of the ani

mals His results are compatible with earlier findings of Stefanopoulo an Callinicos (1932) and of Snijders Postmus and Schuffner (1931) Mor recent studies by the writer have shown that the sera of monkeys immune to yellow fever virus may neutralize dengue but only to a degree that is not generally regarded as being significant Sera of human beings and of experimental animals immune to a great variety of diseases as the result of either natural or experimental infection have been examined in the yellow fever protection test without the discovery of any cross immunity Nearly twenty years use of the mouse protection test in extensive surveys of yellow fever immunity and in a variety of experimental studies by many competent investigators leads to the conclusion that this test ranks very high in specificity and efficiency among the biologic reactions currently in use

CHOICE OF A METHOD FOR PROTECTION TESTS

The more recently developed methods for performance of the mouse protection test offer greater sensitivity in the detection of antibody than the original method proposed by Sawyer and Lloyd (1931) However in the conduct of immunity surveys the purpose of which is to define the limits of spread of the disease it may be more important to exclude with certainty all nonimmunes than to determine the exact percentage of immunity in case the disease has occurred in the area concerned In such cases it is unnecessary to employ a very sensitively balanced test and it may even be unwise to do so However in other instances it may be highly important that he most sensitive methods available be used For example in studying the action to vaccination with a view to determining the time when immunity first demonstrable in comparing the reaction of groups of individuals to fferent vaccine preparations or in testing the reaction of wild animals to perimental infection the principal objective may be the detection of imal quantities of antibody In such cases a sensitively balanced intra bral (Bugher 1940) or intraperitoneal (Whitman 1943 Smithburn) technique would be employed In a choice between the latter tests vailability of infant mice of known age as required for the more sensi ntraperitoneal techniques or of appropriately assayed desiccated virus quired for the intracerebral test might be the deciding factors

COMPLEMENT FIXATION REACTION

Frobisher (1929), attempting to discover a method for the in vitro performance of tests for immunity to yellow fever, prepared an antigen according to a technique used earlier by Hindle (1929) in experiments on vaccination and employed this antigen in complement fixation tests. Portions of liver and spleen from monkeys dying of yellow fever were triturated with sand and half their weight of 9.0 per cent sodium chloride was added. After this preparation had stood overnight in the refrigerator, sufficient distilled water to bring it to physiologic concentration was quickly added and the mixture was centrifuged and passed through Berkefeld V filters. The filtrate was used as antigen. Similar antigen was prepared from tissues of noninfected animals and used as a control. Some nonspecific reactions were observed in that the normal antigen occasionally fixed complement in the presence of yellow fever antisera and some sera from nonimmune individuals reacted with the yellow fever antigen. Nevertheless, 18 of 23 sera from animals known to be immune to yellow fever reacted positively, indicating that if better reagents could be obtained the test might be useful for survey and other purposes.

This antigen was found by Frobisher to contain active virus. If its reactivity depended on the viability of the virus, some of its potency must have been destroyed by the overnight exposure to 9.0 per cent sodium chloride, as this and other salts have deleterious effects upon the virus.

Moses (1929) demonstrated the presence of complement fixing antibodies in the blood of patients ill with yellow fever and found that the coctoantigen employed in the test is antigenic in rabbits. His observation that the most intense reaction occurred with sera taken on the 5th or 6th day suggested that the antibody might not be permanently retained.

In further studies Frobisher (1931*a* and *b*) found that active antigens could be prepared in a variety of ways. The best method (Frobisher, 1931*c*) involved the desiccation in vacuo over sulfuric acid of triturated liver tissue from infected monkeys, followed by extraction with ether in a Soxhlet apparatus for 8 or more hours. The desiccated fat-free residue was stored at room temperature in sealed containers. The desiccated material could be rehydrated, extracted with physiologic saline and filtered to yield an effective antigen at any time during several months after preparation.

This antigen was quite active had the distinct advantage of being non infective and was at least as specific as any prepared previously

G E Davis (1931) meanwhile made a comprehensive study of the techniques and applications of the complement fixation test in studies on sera of monkeys and *human beings in West Africa He failed to obtain* complement fixation with convalescent monkey serum tested against the coctoantigen prepared by Moses (1929) but confirmed the activity of antigen *extracted by Frobisher s* original method although finding that it was *active only in low dilutions* In searching for a better antigen he employed a 1 in 96 dilution of citrated plasma taken from a monkey at the onset of its febrile response to yellow fever virus This material gave good fixation in dilutions up to 1 in 80 with convalescent monkey sera but did not react with normal monkey or normal human serum It was found that serum or plasma virus antigen could be incubated for 15 minutes at 55°C—which was sufficient to render it noninfective—without appreciable loss of antigenicity *In a series of well controlled experiments employing heat inactivated serum* virus as antigen Davis observed that infected monkeys regularly developed complement fixing antibodies during convalescence and that the production of antibodies was specifically dependent upon active infection Inoculation with inactive virus did not give rise to the formation of the antibody and the administration of immune serum together with or followed by active virus elicited antibody formation only when there was a resultant thermal reaction to the virus The antibody could be demonstrated within a few days after defervescence but its disappearance within a few months was regularly observed In monkeys from which the antibody had disappeared the original or a higher titer could be restored by inoculation of fresh or *heat inactivated serum virus—the anamnestic* phenomenon It is of interest to note in this connection that although inactivated virus was incapable of evoking antibody formation it could recall the antibody in an animal that *had formerly produced it*

Davis employed the inactivated serum virus antigen in tests with sera from 27 persons who had recovered from illnesses diagnosed as yellow fever Twenty one of the sera tested had previously been examined in the monkey protection test and all were known to be protective but only six fixed complement—five of these very weakly With a group of sera from persons residing in an infected area 40 per cent of which were protective to monkeys uniformly negative results were obtained in the complement fixation

test Davis therefore concluded that the test is valueless in surveys of immunity among human beings

Hudson (1931*b*) applied the monkey protection test and the complement fixation test to a study of the sera of five persons who had contracted yellow fever in the laboratory The antigen used in this study was desiccated infectious monkey serum Although protective antibodies appeared on the 5th to the 7th day in the sera of each of the four persons tested and persisted throughout the experiments in the sera of all five complement binding antibodies appeared later and in one person were never demonstrated during 8 months after the illness In later studies Hudson (1932) noted that only four of seven sera from human convalescents fixed complement When the sera of monkeys recovered from active infection were used the results were regularly positive but sera of monkeys surviving in protection tests and sera of vaccinated monkeys usually gave negative reactions In his report on these studies Hudson stated in conclusion The complement fixation test in our hands was not an effective means of determining the establishment of immunity in vaccinated monkeys

Frobisher (1931*b* and *c*) found that a certain percentage of human sera reacting positively in protection tests gave negative results in complement fixation tests G F Davis (1931) obtained even less satisfactory results with human sera although his results with monkey sera were fairly satisfactory Soper Frobisher et al (1932) made a postepidemic survey of immunity employing both the monkey protection test and the complement fixation reaction They found a high degree of correlation between results obtained by the two methods but observed that there is considerable tendency for early positive complement fixation tests to become negative They concluded that complement fixing bodies demonstrable by present methods are less constantly produced than are protection bodies and are less permanent Nevertheless they believed that the complement fixation test might be useful in field studies made soon after an epidemic

Soper and de Andrade (1933) made a postepidemic survey in the Cambucy area of Brazil employing both the intraperitoneal mouse protection test and the complement fixation test in examination of sera They found a higher percentage of positive complement fixation reactions 6 to 10 weeks after attacks of the disease than before or after that time indicating again that the complement binding antibodies tend to appear later and to disappear earlier than the protective antibodies

Lennette and Perlowagora (1943) undertook a study of the complement

fixation test in yellow fever employing a mouse brain antigen prepared by the method of Casals and Palacios (1941) They observed as had Frobisher a tendency for Wassermann positive sera to give nonspecific reactions but found that these could be eliminated in many instances through inactivation of the sera by heat They also made the important observation that human beings inoculated with 17D vaccine do not ordinarily develop complement fixing antibody and pointed out that this fact might be used in immunity surveys to distinguish vaccinated persons from those immune as a result of having had the disease However a positive protection test and negative complement fixation test might indicate either a vaccinated person or one who had recovered from the disease sufficiently long before to have lost his complement fixing antibody

Fox Lennette et al (1942) confirmed the observation that inoculation with the 17D vaccine is not ordinarily followed by the development of complement fixing antibody but they also found that the antibodies were produced with considerable regularity in persons who exhibited encephalitic symptoms and signs following receipt of certain batches of the vaccine The encephalitogenic property of the vaccine was ascribed to the occurrence of unexpected and unpredictable variation of the 17D virus at a certain passage level as a result of which the virus possessed enhanced neurotropic pathogenicity for man

Perlowagora and Lennette (1944) investigated the usefulness of the complement fixation reaction as a diagnostic method They employed known reacting sera (antibody) against the sera of monkeys infected experimentally (antigen) and found that active antigen was present during the illness in monkeys having febrile reactions or in those having fatal infections with or without fever In nonfatal infections in which there was no febrile reaction the antigen was not demonstrable They concluded that the antigenicity must be referred not to the virus per se but to some substance resulting from the reaction provoked in the host s tissues by the virus In a continuation of these studies (Lennette and Perlowagora 1945) they noted a great variation in the extent of lesions in marmosets dying of yellow fever They stated that in some instances diagnosis of the disease could not be established by histologic examination alone and that the test for specific antigen in the tissues of the infected animal was a more reliable diagnostic method

Returning to the use of the complement fixation test for survey purposes Perlowagora and Hughes (1947) prepared an antigen from the globulin

fraction of the ether extracted brains of mice infected with French neurotropic yellow fever virus This they found to be equal or superior to previously prepared antigens both in sensitivity and in specificity With it they found that complement fixing antibodies are formed in mild and subclinical infections with yellow fever virus but not often following vaccination thus reaffirming the usefulness of the test in the detection of recent natural infections In a later study Perlowagora and Hughes (1948) observed that experimentally infected monkeys develop complement fixing antibody but regularly lose it within a year Thus in surveys either in man or in wild primates a positive serum complement fixation reaction is indicative of recent natural infection probably within the year

All the available information indicates that the complement binding antibody is separate and distinct from the neutralizing or protective antibody It seems likely however that the complement binding antibody is the same as the precipitating antibody (see below)

PRECIPITIN TESTS

Frobisher (1932) sought to obtain information regarding the chemical nature of the virus of yellow fever and to find a simple procedure for early diagnosis of the disease by means of precipitin tests He inoculated rabbits repeatedly with relatively large doses of filtered suspensions of infected aegypti mosquitoes but was not able at any time to demonstrate precipitin in their sera when these were overlaid with virus-containing sera of infected rhesus monkeys Neither was protective antibody demonstrable in the rabbit sera Conversely the sera of rabbits inoculated over a period of 6 weeks with repeated doses totaling 20 0 to 31 5 cc of serum taken from rhesus monkeys at the first access of fever following inoculation with yellow fever virus were tested against filtrates of suspensions of infected aegypti no precipitins were demonstrable although protective antibodies were present Previously Frobisher (1930) had obtained no precipitation with immune sera tested against eight antigens from urine alcoholic extracts of livers and hearts acetone insoluble extracts of livers and hearts and saline extracts of livers and spleens from infected monkeys In view of these results it appeared that the hopes of developing a precipitin test were unwarranted

Hughes (1933) approached the problem in a different manner and obtained encouraging results He employed as antigen the serum of a rhesus monkey dying of yellow fever carefully depositing this serum above the

serum of an immune monkey The preparation was incubated for 2 hours at 37°C and examined under artificial light against a dark background Heavy turbidity developed becoming maximal at the end of the incubation period Normal monkey serum mixed with either of the preceding reagents failed to show precipitation Similar precipitinogen was demonstrated in the sera of 53 of 54 monkeys bled when moribund or at autopsy and in sera of 15 of 26 of the same monkeys taken at the onset of fever Five different strains of virus each highly virulent for rhesus monkeys had been used for the inoculation of these animals yet the results indicated no strain specificity Four other monkeys were inoculated with the F W strain of virus which is much less virulent for monkeys Sera from two of these animals showed small amounts of the precipitinogen for 1 or 2 days only—the 1st and 2d day after the onset of fever sera from the other two showed none at any time Three monkeys that suffered nonfatal infections following inoculation of the highly virulent Asibi virus each exhibited precipitinogen the agent was not demonstrable in the urine and was present in the liver only in quantity accountable for by the content of the blood The sera of monkeys inoculated with virulent pantropic virus together with known immune serum in sufficient quantity to prevent death showed no antigen and blood samples drawn 4 weeks later did not contain precipitating antibody It was found that the quantity of precipitinogen did not parallel the virus content of the serum and precipitinogen could be demonstrated in high titer in samples taken post mortem in which protective antibody was demonstrable A virus-containing serum stored until virus was no longer demonstrable was found still to contain precipitinogen and upon injection into a monkey evoked the formation of precipitating antibody Thus the precipitinogen was found to be not a hapten but a true antigen

Inoculation of neuroadapted yellow fever virus into monkeys was followed by circulation of the virus in large quantities and the formation of protective antibodies but neither precipitinogen nor precipitin was demonstrated in the same serum samples A monkey immune as a result of inoculation with neurotropic virus and possessing protective but not precipitating antibodies developed the latter upon inoculation with precipitinogen-containing serum Specimens of serum which contained active precipitinogen remained active after sufficient heating to render them nonpathogenic However boiling destroyed the precipitinogen The antigen was not precipitated by half saturated ammonium sulfate but was associated with the precipitate formed when the solution was brought to full saturation

Only 10 human sera were examined by Hughes for precipitin. Five of these reacted, while the remainder did not. Those that reacted gave negative results in tests against normal monkey serum. It was noted that four of the five nonreacting sera were from persons who had had mild infections, while those that exhibited the precipitin were generally from persons who had suffered the more severe infections. Mention was made that most of the monkeys that exhibited precipitin lost this antibody after a few months, and it is possible that some of the nonreacting human beings had possessed the antibody earlier.

The precipitinogen appeared to be related to the complement fixation antigen and the precipitin to the complement binding antibody.

From the studies discussed, it is evident that the precipitin test would be of little value for retrospective diagnosis or survey purposes, but it is possible that it might be a useful diagnostic aid, particularly in severe cases. Further work on the subject seems to be indicated.

SEARCH FOR OTHER IN VITRO IMMUNOLOGIC METHODS

Several workers have sought methods of performing immunologic tests for yellow fever that would not require the use of animals. Most of their results remain unpublished for the reason that no wholly reliable method has been found. If such a method could be perfected the detection of antibody or virus might be accomplished more speedily and more cheaply.

Following a lead suggested by the work of Cannon and Marshall (1940) and of Weir (1941), who worked with nonviral antigens, Goodner (1941) sought to enhance and render visible the reaction between virus and antibody by the absorption of the virus-antibody complex on collodion particles. He reported a visible reaction with human immune serum but noted that the sera of rabbits and monkeys failed to react specifically. Weir employed a different technical approach, absorbing virus on collodion particles and studying the agglutinative effect of the addition of serum. Many sera containing antibody reacted visibly, yet others did not, so that the method was not found applicable to field use. It would seem that further investigation along these lines is indicated.

TRANSMISSION OF IMMUNITY FROM MOTHER TO OFFSPRING

Studies made at the Yellow Fever Laboratory at Bahia, Brazil, first revealed that infant rhesus monkeys born to immune mothers exhibit protective antibody against yellow fever virus in their sera (Hoskins 1934a). Sera of five infants bled 1 to 5½ months after birth showed the presence of the antibody, as did specimens from the mothers. Two of the five were bled again when they were 8½ months of age and 3 weeks after they were weaned. Both sera were then lacking in antibody. Four other infants born of immune mothers were bled for protection tests within 12 hours of birth and the serum of each was found to contain neutralizing antibody. The latter observation suggested that the antibodies are derived from the mother through the placenta but did not prove this beyond question, as it was not definitely known that the infants had not suckled.

Stefanopoulo, Laurent, and Wassermann (1936) demonstrated the presence of neutralizing antibodies against yellow fever virus in the breast milk of women immune to the disease, and there was a tendency for a time toward the belief that the antibody in the offspring is derived from the mothers by suckling. However, studies made in West Africa and reported by Soper, Beeuwkes, et al. (1938) showed conclusively that the antibody is present before nursing has taken place in human infants born to immune mothers. Seventeen of 19 blood specimens taken from the umbilical cords of infants born of immune mothers were protective, whereas each of 61 specimens from the offspring of nonimmune mothers was nonprotective. It was also found by the same investigators that the antibody may disappear from the serum of the infant while it is still nursing. The longest demonstrated persistence of the inherited immunity was 5½ months in rhesus monkeys and 6 months in children. Susceptibility tests made in young monkeys following the disappearance of the antibody resulted either in their death or in febrile infection with recovery associated with the development of an active immunity.

The transmission of a transitory immunity from the immune mother to her offspring has been demonstrated in many diseases of both human beings and animals. In some species the transmission probably occurs transplacentally and in others through the colostrum, depending on the intimacy of association of maternal and fetal bloods in the species concerned. By

whichever route the antibody may be acquired in the first instance, it is certain that the continued suckling of an immune mother does not result in persistence of the immunity in the offspring Furthermore, it is clear that the factors that are responsible for the enduring character of the immunity in the mother are at no time transmitted to the infant, since the antibodies do not remain long in the blood of the latter

The occurrence of antibody in the serum of infants born to immune mothers must be taken into consideration in conducting surveys of immunity to yellow fever, among either human beings or wild animals Sampling of individuals of an age at which this phenomenon occurs is not widely practiced, but specimens are obtained on occasion from very young individuals For example, in acquiring monkeys, or samples of blood from them, for survey purposes, it occasionally happens that an infant is obtained without its mother being captured If such an individual is under 6 months of age, the fact that its serum is protective does not necessarily indicate that the animal itself has been infected

The phenomenon may have additional epidemiologic significance with reference to the question of whether or not very young infants require vaccination If the mother were immune, her offspring would probably have sufficient immunity to result in protection for some weeks or a few months However, the time of disappearance of the antibody is variable and its presence can be determined only by test If the child is living in an endemic area, the administration of the vaccine should not be delayed more than about 2 months, or perhaps not at all during epidemics The presence of the antibody in the infant s blood would render the vaccine ineffectual yet to withhold the vaccine might be hazardous in the event that the antibody had disappeared

INDUCED IMMUNITY

INEFFICIENCY OF INACTIVATED VIRUS AS VACCINE

Although a number of investigators had failed in their efforts to produce a vaccine by chemical treatment of the virus (Pettit and Stefanopoulo 1928, Aragão, 1928, Hindle, 1929, Monteiro 1930) further exploration of this field was undertaken by International Health Division workers Burke and Davis (1930) reported a fatal case of yellow fever in a laboratory worker who had been inoculated 5 months previously with a liver vaccine ' This

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The transmission of a transitory immunity from the immune mother to her offspring has been demonstrated in many diseases of both human beings and animals. In some species the transmission probably occurs transplacentally and in others through the colostrum, depending on the intimacy of association of maternal and fetal bloods in the species concerned. By

whichever route the antibody may be acquired in the first instance, it is certain that the continued suckling of an immune mother does not result in persistence of the immunity in the offspring. Furthermore, it is clear that the factors that are responsible for the enduring character of the immunity in the mother are at no time transmitted to the infant, since the antibodies do not remain long in the blood of the latter.

The occurrence of antibody in the serum of infants born to immune mothers must be taken into consideration in conducting surveys of immunity to yellow fever, among either human beings or wild animals. Sampling of individuals of an age at which this phenomenon occurs is not widely practiced, but specimens are obtained on occasion from very young individuals. For example, in acquiring monkeys, or samples of blood from them, for survey purposes, it occasionally happens that an infant is obtained without its mother being captured. If such an individual is under 6 months of age, the fact that its serum is protective does not necessarily indicate that the animal itself has been infected.

The phenomenon may have additional epidemiologic significance with reference to the question of whether or not very young infants require vaccination. If the mother were immune, her offspring would probably have sufficient immunity to result in protection for some weeks or a few months. However, the time of disappearance of the antibody is variable and its presence can be determined only by test. If the child is living in an endemic area, the administration of the vaccine should not be delayed more than about 2 months, or perhaps not at all during epidemics. The presence of the antibody in the infant's blood would render the vaccine ineffectual; yet to withhold the vaccine might be hazardous in the event that the antibody had disappeared.

INDUCED IMMUNITY

INEFFICIENCY OF INACTIVATED VIRUS AS VACCINE

Although a number of investigators had failed in their efforts to produce a vaccine by chemical treatment of the virus (Pettit and Stefanopoulo, 1928, Aragão, 1928, Hindle, 1929; Monteiro, 1930), further exploration of this field was undertaken by International Health Division workers. Burke and Davis (1930) reported a fatal case of yellow fever in a laboratory worker who had been inoculated 5 months previously with a "liver vaccine." This

report did not state how the vaccine had been prepared but Sawyer Kitchen and Lloyd (1932) mentioned that the infected liver tissue had been treated with formalin and phenol N C Davis (1931*c*) attempted to induce immunity to yellow fever by the inoculation of chloroform extracted livers of infected monkeys No immunity was induced by such preparations when the exposure to chloroform was sufficient to render the material avirulent

Sawyer Kitchen and Lloyd (1932) gave a summary of a number of experiments with the use of vaccines prepared by exposure of the virus to (*a*) a temperature of 37°C (*b*) the effects of formaldehyde or tricresol at room temperature or (*c*) the effects of tricresol at 37 or 0 5°C They stated

> As a rule the vaccines exposed for the shortest periods to the attenuating agents produced yellow fever in the monkeys inoculated and the specimens long exposed failed to immunize Between these extremes there were found in some cases exposures which attenuated the virus to such an extent that the vaccinated animals showed no fever but acquired an active immunity This zone in the series of exposure times was inconstant and narrow

These authors accordingly turned their attention to other methods which will be discussed later

Gordon and Hughes (1936) gave details of a series of carefully controlled experiments in which they employed as vaccines either neurotropic or pantropic yellow fever virus treated with heat ultraviolet light or formaldehyde They tested the pathogenic properties of each vaccine in mice and made tests of immunizing potency in monkeys Some of their preparations that were noninfective for mice produced fatal yellow fever when inoculated into monkeys All the preparations containing viable virus caused either death or febrile illness with active immunity upon recovery whereas none of the vaccines that were nonpathogenic exhibited any immunizing power These workers observed that under the condition of their experiments yellow fever virus exposed to ultraviolet irradiation rapidly lost potency and might be wholly inactive within 30 minutes but some preparations contained viable virus after much longer exposure One vaccine made by exposure of pantropic virus under the lamp for 4 hours failed to infect mice upon intracerebral inoculation Two rhesus monkeys were inoculated with large doses of this vaccine One showed neither fever nor circulating virus following the inoculation exhibited no antibodies in its serum 30 days later and succumbed to yellow fever when given a challenge inoculation The other monkey receiving this irradiated vaccine exhibited circu

lating virus in its blood from the 2d day following the inoculation until its death from yellow fever which occurred on the 6th day

It is possible that substances other than virus in the usual media in which yellow fever virus suspensions are contained (mouse brain monkey serum chick embryo) may on the one hand serve to protect portions of the virus from the deleterious effects of physical and chemical agents so that minimal quantities of viable virus escape the treatment or on the other hand by absorption or chemical action or otherwise necessitate a much greater exposure to the inactivating agent than would be required if the virus could be isolated in pure form However that may be it suffices to say that as yet no chemical or physical method has been found for eliminating the pathogenic and at the same time retaining the antigenic properties of the virus

INOCULATION OF ACTIVE MODIFIED VIRUS TOGETHER WITH IMMUNE SERUM

The first use of this method of immunization was reported by Sawyer Kitchen and Lloyd (1931 1932) In principle it consists in the inoculation of a strain of virus whose pantropic properties have been reduced or eliminated by laboratory methods together with sufficient immune serum to prevent the virus from entering the circulating blood of the inoculated subject The authors first made comprehensive experiments in monkeys from which they found that the method is both effectual and safe Thereafter the combined antiserum vaccine was given to 16 persons No ill effects of any consequence resulted and all the individuals developed protective antibody following the inoculations The virus employed was the 105th to 176th passage neurotropic virus of the French strain It was prepared for use in the following manner suspensions usually of 10 per cent concentration of brains of infected mice were made up in whole human serum which was taken in all instances except one from persons immune to yellow fever The suspensions were clarified by centrifugation and in some instances by filtration following which they were frozen and desiccated The immune sera used for preparation of the vaccines and for inoculation were from persons who had had yellow fever or had developed protective antibody against the virus following vaccination Each serum was subjected to preliminary titration in rhesus monkeys and the amount per kilogram of body weight required to protect against pantropic virus was determined The dose of virus given

to human subjects was originally 0 003 gm per kilogram of weight When sufficient information had been acquired on the keeping qualities of the dry virus and on its immunizing power in human beings the dose was no longer varied according to the subject Thereafter 0 5 cc of the rehydrated virus (stored not more than 6 months in a refrigerator in the dry state) was given to each person The dose of yellow fever immune serum given was usually 0 3 cc per kilogram of body weight *but two persons received larger* quantities

Each of the 16 persons exhibited neutralizing antibodies in his serum in 7 to 21 days (No clear cut negative results in mouse protection tests were obtained with sera taken more than 10 days after the inoculations) No serious reactions followed the inoculations and the introduction of the method for use in the immunization of persons doing laboratory or field work on yellow fever brought to an end the long series of laboratory infec tions (Burke and Davis 1930 Berry and Kitchen 1931) that had taken such heavy toll No evidence was found to indicate that this method of vac cination gave rise to the presence of the virus in the circulation of the inocu lated individual Nevertheless a leukopenia was observed in several of the individuals and the authors believed from this and other evidence that the acquired immunity resulted from mild infection

A similar method employing virus modified by in vitro cultivation was proposed by Lloyd (1935) The virus he used was originally pantropic but after cultivation through 130 passages in a medium containing minced mouse embryonic tissue it had lost its power to produce hepatic lesions in monkeys This attenuation was associated with no change in the incubation *period in mice indicating that the virus was less neurotropic than the mouse* adapted virus used by Sawyer Kitchen and Lloyd The dose of the com bined vaccine given by Lloyd was 25 000 to 170 000 mouse lethal doses of virus together with 0 5 or 0 6 cc of human immune serum per *kilogram* of body weight The results reported were similar in all respects to those reported by Sawyer Kitchen and *Lloyd (1932)* except *that such reactions* as occurred took place a little earlier

Theiler and Whitman (1935*a*) made a quantitative study to determine the amount of serum required to prevent the neurotropic virus from ap pearing in the circulation following combined inoculation In one of their experiments six monkeys were inoculated extraneurally with a dose of neu rotropic virus calculated as two mouse infective units Three of the mon keys were given sufficient doses of immune serum to prevent circulation of

the virus while the other three received such small doses of serum that the virus was demonstrable repeatedly in daily tests. Each of the latter three developed encephalitis. In the same experiment six other monkeys were given 200,000 mouse infective doses of the virus and doses of serum varying from 2.5 to 0.0008 cc. per kilogram of body weight. The four animals of this group receiving the smaller doses of serum each exhibited circulating virus but none of the six developed encephalitis. All became immune. Thus it was shown that a small dose of neurotropic virus (with inadequate immune serum) is more likely to give rise to encephalitis than is a large dose and that encephalitis does not occur if the dose of immune serum is sufficient to prevent the virus from entering the circulating blood. An excess of immune serum, given with the virus, did not prevent the development of active immunity. This work established a method for determining the minimum quantity of any serum that could be used with safety in the combined vaccination.

While the method of immunization with virus together with immune serum was most useful in that it ended the very great hazard to which workers engaged in yellow fever investigations were subject, it was not practical for field application owing to the large quantities of immune serum required. Hyperimmune sera had by this time been produced in several different species of animals (in horses by Pettit and Stefanopoulo, 1933; in rabbits by Whitman, 1935; in goats by Hughes and Lloyd, quoted by Lloyd, 1935; in monkeys by Theiler and Smith, 1936), but it was found by Soper and Smith (1938*a*) that the use of hyperimmune goat serum not only evoked serum reactions but also permitted the occurrence of infection, owing to rapid inactivation of the heterologous antibody. Moreover, the cost of immune or hyperimmune serum for use in mass vaccination would be prohibitive. The principal value of the method of vaccinating with modified virus plus immune serum probably lay in the fact that it prevented yellow fever among the professional and technical staff who went on to develop another vaccine which was applicable to mass usage.

INOCULATION WITH ACTIVE NEUROTROPIC VIRUS WITHOUT IMMUNE SERUM

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lar method was used by Aragão (1933*a*) Untoward reactions were encountered with this type of vaccine and Laigret (1934) accordingly proposed and used in the inoculation of a considerable number of human beings a vaccine consisting of neurotropic yellow fever virus attenuated by exposure to a temperature of 20°C Three injections of virus exposed to this temperature for 4 days 2 days and 1 day respectively were given at 20 day intervals Findlay (1934*b*) made the significant observation that inoculation with this vaccine and the *resultant development of immunity is correlated with* the entry of the virus into the circulation of the vaccinated subject Theiler and Whitman (1935*b*) showed that the treatment of the virus as proposed by Laigret does not in fact result in attenuation but only in a progressive decline in the quantity of living fully virulent virus present in the preparation and that the method therefore consists in the administration of graded doses of fully virulent neurotropic virus They emphasized the potential hazards attendant upon use of such material in particular for children.

In the experience of different workers inoculation with the neurotropic virus without immune serum yielded varying results some of them unfavorable Another modification of the vaccine was proposed which involved incubation of glycerolated virus the addition of disodium phosphate and further incubation and ultimate incorporation of the desiccated material in egg yolk Vaccine thus prepared was administered in single doses by Mathis Durieux and Mathis (1936) to 150 persons in French West Africa Among the 50 Africans included in the inoculated group no reactions to the vaccine were noted Observations were made in 376 Europeans who received the vaccine and of this number 39 per cent were recorded as having mild moderate or prolonged reactions characterized by headache fever and in some patients nervous symptoms or jaundice No severe reactions were reported and no deaths occurred.

Further investigations on the use of neuroadapted virus as a vaccine notably by French workers led to the development of the scratch method *of administration* which will be discussed later In the light of present knowledge it seems probable that much of the apprehension and perhaps most of the untoward results occasioned by injection of the neurotropic virus for purposes of immunization were due to the fact that the virus used was at relatively low passage levels and hence may not have been as fixed as it became later with continued animal passage

IMMUNIZATION WITH THE 17D ATTENUATED VIRUS BY INJECTION

Lloyd Theiler and Ricci (1936) reported the results of a comprehensive series of experiments on the in vitro propagation of yellow fever virus The Asibi pantropic virus and the neuroadapted French virus of high and low mouse passage levels were cultivated in various media and underwent various modifications in their pathogenic properties Further studies of the changes in pathogenic properties of three lines of tissue culture virus each derived originally from the Asibi strain were made by Theiler and Smith (1937*a*) One of these the 17D strain was cultivated first for 18 passages in minced mouse embryo Tyrode s solution medium then for 58 generations in whole chick embryo Tyrode s solution followed by 160 subcultures in Tyrode s solution with chick embryo from which the head and spinal cord had been removed It then exhibited a loss of viscerotropism without enhancement of neurotropism and possessed good antigenic properties This virus was first employed for immunization of human beings by Theiler and Smith (1937*b*) and was found to evoke a satisfactory antibody response without eliciting serious postvaccinal reactions The first field use of this virus as vaccine was reported from Brazil by Soper and Smith (1938*b*) and Smith Penna and Paoliello (1938) It was observed that the injection of 17D vaccine induced an effective immunity within 1 week of inoculation among persons residing in an epidemic area although neutralizing antibodies usually did not appear until after the 7th day Such reactions to the vaccine as occurred were mild and no contraindications to its administration were found (Fig 25)



Fig 25 One hundred doses of 17D yellow fever vaccine with its diluent ready for use

Smith, Penna, and Paoliello (1938) described the mode of preparation of the vaccine and the methods employed in safety control and potency tests They reported the inoculation of 59,107 persons, protection tests on 882 of whom showed 94 1 per cent to be immune following the vaccination In an appendix they listed all the equipment used by the field teams who administered the vaccine and discussed the practical problems encountered The report of Soper and Smith (1938*b*) covered the further use of the vaccine during 1937 and 1938, when more than half a million persons were inoculated

Mass vaccination was continued in Brazil, not only in zones where the infection was known to exist, but also in areas where occurrence of the disease was anticipated, owing to its wavelike spread in that country Immunization by the same method was practiced also in England, Colombia the United States, various countries in Africa and, during the Second World War, in the armed forces of the Allies on a very large scale

Mild reactions, which rarely incapacitate the individual, take place in a small proportion of persons receiving the 17D vaccine More serious reactions of two types have occurred, but the cause and means of prevention of both have been found Fox, Lennette, et al (1942) reported the occurrence of encephalitis among some of the persons receiving certain lots of the vaccine in Brazil They noted that, whereas persons inoculated with the 17D vaccine do not, as a rule, develop complement fixing antibodies, a considerable proportion of those who had encephalitis did produce these antibodies Theiler (p 109 of this volume) and Fox and Penna (1943) have shown that at certain passage levels, variations in the pathogenic properties of the 17D virus may take place in consequence of altered cultural environment, although Smith, Calderon Cuervo, and Leyva (1941) had found no significant difference in immunizing potency of high and low culture passage virus Since the establishment of a seed lot system in the production of vaccine, with the result that the passage level changes little, there has been no further occurrence of encephalitis in inoculated persons

While vaccination with mouse adapted virus in combination with immune serum was in practice, cases of icterus following inoculations were reported from both England (Findlay and MacCallum, 1937*b* and 1938) and Brazil (Soper and Smith, 1938*a*), and it was believed that the causative factor of the jaundice was probably associated with the serum rather than with the yellow fever virus Following the adoption of the 17D virus, and after the use of immune serum in association with the vaccine had been

discontinued. Fox, Manso, et al. (1942) reported an outbreak of icterus among persons vaccinated in Brazil in 1939. It should be noted, however, that the vaccine then in use still contained nonimmune human serum as a menstruum for the virus-infected chick embryos. Since the possibility could not be ruled out that the icterogenic agent was associated with the human serum, great care was taken in the selection of persons who served as donors of serum used by The Rockefeller Foundation staff in preparation of the vaccine. Moreover, the serum was "heat inactivated" prior to use in the belief that any pathogenic agent that might have been contained therein would thus be destroyed before the serum was mixed with the chick embryos.

With the entry of the United States into the Second World War and the subsequent widespread use of the 17D vaccine for immunization of military personnel, a major outbreak of hepatitis occurred among persons receiving certain lots of the vaccine (Sawyer, Meyer, et al., 1944; Turner, Snavely, et al., 1944). All the evidence obtainable indicated that the icterogenic factor was an unusually thermostable filtrable virus derived from the pooled nonimmune human serum used as a medium in the preparation of the vaccine (Findlay and Martin, 1943). Prior to this time experimental lots of 17D vaccine had been prepared by International Health Division workers, using distilled water as the diluent. The vaccine thus prepared had been found to possess both adequate immunizing potency and suitable stability. Therefore, when the outbreak of hepatitis occurred, the use of serum as a menstruum for the chick-embryo virus was dispensed with, and sterile distilled water was thereafter used instead. Hargett, Burruss, and Donovan (1943), working independently, also found that satisfactory vaccine could be prepared with an aqueous base. This mode of preparing the material has since been used exclusively and without further occurrence of hepatitis.

Prior to its adoption for the immunization of human beings, a great deal of experimental work was done with the 17D virus for the purpose of studying its pathogenic and antigenic properties. The studies, in particular those pertaining to the antigenicity, were continued after the virus was in widespread use. The virus has to be regarded as a variant since its pathogenic properties, in the aggregate, differ from those of unmodified pantropic strains and from neuroadapted lines of yellow fever virus. Moreover, the procedures that gave rise to its origin from the highly virulent Asibi virus, when repeated, did not again result in equivalent modification. A factor or factors unknown were therefore responsible for the observed alteration in pathogenic properties.

The earliest studies made with yellow fever virus in experimental animals (Stokes Bauer and Hudson 1928*b*) showed that the inoculation of a monkey with unmodified virus gives rise if death does not ensue to the development of immunity associated with the presence of neutralizing antibodies in the blood When the hepatotropic affinities of the virus were eliminated through serial intracerebral passage in mice (Theiler 1930*b*) it was found that the capacity of the resulting neuroadapted virus to induce immunity was unaltered and that the antibodies produced were effective against both neurotropic and pantropic virus Likewise it was noted (Theiler and Smith 1937*a*) that although the 17D virus had lost both the capacity to cause lesions in the livers of susceptible animals and for the most part the capacity to cause encephalitis when inoculated intracerebrally into monkeys it nevertheless evoked the formation of neutralizing antibodies Theiler and Smith (1937*b*) found that although no antibody was demonstrable in the serum of monkeys 7 days after vaccination with 17D virus the animals were already solidly immune to challenge inoculation with fully virulent pantropic virus Subsequent studies (Smithburn and Mahaffy 1945) showed that effective immunity is present in rhesus monkeys within 5 or 6 days of vaccination and that if protection tests sufficiently sensitive in method are employed neutralizing antibody is demonstrable in the sera of the monkeys within 6 or 7 days of the inoculation The experiments of Theiler and Smith (1937*b*) and of Smithburn and Mahaffy (1945) together with certain additional experiments performed by Theiler employing rhesus monkeys in studies of the immunizing potency of 17D vaccine are brought together and summarized in Table 5 From these data it will be seen that none of 16 monkeys challenged with Asibi virus 6 or more days after inoculation with the 17D virus exhibited circulating virus following the challenge inoculation and all of them survived Furthermore the data show that of 12 monkeys receiving challenge inoculations 5 days after vaccination none died although six of them exhibited circulating virus on one or more days

In man the appearance of demonstrable antibody in the serum following inoculation of 17D vaccine is slightly delayed however it is present by the 10th day in a high percentage of vaccinated persons (Smithburn and Mahaffy 1945) Antibody production may continue in man for 3 to 4 weeks

Failure of development of neutralizing antibody in rhesus monkeys following inoculation with 17D virus of proved potency has not been observed

TABLE 5

RESULTS OF CHALLENGE INOCULATIONS IN RHESUS MONKEYS VACCINATED WITH 17D VIRUS AND IN NONVACCINATED CONTROLS

No. of monkeys	LD_{50} 17D virus as vaccine	Interval between vaccination and challenge	LD_{50} Asibi virus, challenge	Circulating virus	Fate of monkey	
					Died	Lived
		(days)		(days)		
14	3 841 to 100 000	7 to 14	82 to 10 000 000	0	0	14
2	6 513	6	121 500	0	0	2
2	100,000	5	82	0	0	2
1	16 500	5	10 000 000	2–4	0	1
2	7,869	5	121 500	0, 3	0	2
2	2 600	5	113	0	0	2
3	430	5	480	4–6, 5, 2–4	0	3
2	260	5	113	0 2	0	2
2	100 000	3	82	3 5 2–6	1	1
1	16 500	3	10 000 000	2–4	1	0
1	13 861	3	121 500	2 5	0	1
2	5 000	3	113	2, 3 5	0	2
3	800	3	480	3, 2–6, 3–6	1	2
2	500	3	113	2 5 2 6	0	2
2	100 000	1	82	1 7 2–6	1	1
1	25 885	1	121 500	1–5	1	0
1	16 500	1	10 000 000	2 3	1	0
2	100 000	<1	82	3–7 4–6	1	1
1	16 500	<1	10 000 000	1 3	1	0
2	0		10 000 000	1 3, 1 5	0	2
2	0		121 500	1–6 2 5	2	0
3	0		480	2 5 3–5, 2–4	3	0
4	0		113	2–4, 2–4, 2–4, 2 5	4	0
2	0		82	1 5 2–4	2	0

Failures of immunization with vaccine of proved potency have occurred among human beings, but these are by no means common In groups of persons who were under close laboratory observation and who received vaccine of proved potency Theiler and Smith (1937*b*) and Smith, Penna, and Paoliello (1938) found that all developed antibody as a result of their inoculations In a field study controlled by a preinoculation survey of immunity, the latter investigators noted an increase in the incidence of im-

munity from 1 67 per cent to 98 37 per cent in six localities where vaccinations were done and the viability tests showed that the vaccine used was potent It is evident, therefore, that the development of neutralizing antibodies occurs with great regularity as a result of inoculation with this virus However, it is worthy of note that Theiler and Smith (1937*b*) found that the quantity of antibody produced as a result of the inoculation may not be very high Various subsequent investigations have shown that the antibody level is usually below that evoked by an attack of yellow fever and that its detection especially at intervals of several years after vaccination, may require sensitive methods (Whitman, 1943, Smithburn, 1945)

Studies on the duration of the antibody response evoked by the 17D vaccine have been made at various intervals after inoculation The longest intervals covered in persons not known to have been exposed to the virus in the interim were 5 and 6 years The results indicate that the induced immunity is well sustained for this period, about 90 per cent of persons showing protective antibodies (Anderson and Gast Galvis, 1947, Dick and Smithburn 1949) Inasmuch as the virus is viable and probably multiplies in most individuals it seems not unlikely that the induced immunity may be lifelong

A further factor of safety with reference to the use of the 17D virus as a vaccine was brought out by the work of Roubaud, Stefanopoulo, and Findlay (1937) and Whitman (1939) They found that this virus (Whitman) and a closely related tissue culture line (Roubaud et al) cannot be transmitted by *A aegypti* Therefore although minimal quantities of the virus may appear in the circulation following inoculation, as has been shown to be the case there is no danger of its getting out of control or undergoing change in pathogenicity through the agency of these insects

DAKAR TOPICAL METHOD OF IMMUNIZATION

Faced with the problem of the control of yellow fever in a vast tropical empire for which their country was responsible, French investigators in particular at the Pasteur Institute in Dakar, have diligently pursued as a long term problem the development of a safe, effective and inexpensive vaccine against yellow fever Inoculation with neuroadapted virus prepared by one method after another fell short of the ideal, and the search continued In 1939, Peltier Durieux et al (1939*b*) reported that the application of an appropriate preparation of neurotropic yellow fever virus to the

kin followed by mild scarification as practiced in smallpox vaccination esulted in the development of immunity to yellow fever They proposed ie combined use of yellow fever and vaccinia viruses by this topical method or immunization against the two diseases in the tropical world The afety of the method from the point of view of possible mosquito trans ission of virus derived from inoculated persons was indicated by the ork of Peltier Durieux et al (1939*a*) who found that aegypti mosquitoes ed upon persons vaccinated in this way with the neurotropic virus rarely maintained the virus and even when they did were unable to transmit it y bite Other workers have obtained successful transmission of neurotropic irus to both monkeys and mice (Davis Lloyd and Frobisher 1932) through he agency of ae. aegypti but such experiments may have involved virus having lifferent pathogenic properties and certainly involved different hosts and hey therefore do not necessarily invalidate the results of Peltier Durieux t al (1939*a*)

Peltier Durieux et al (1940) reported the first extensive field trial of ieurotropic virus applied topically the results of which were satisfactory The work of the Dakar group was pursued throughout the years of the econd World War under difficult circumstances and was given little public ty until the cessation of hostilities In a postwar publication Peltier (1947) eported the vaccination of more than 14 000 000 persons by this method The program as presently conceived involves vaccination of the entire popu ation of the colonies with the two viruses administered simultaneously once very 4 years in order to insure immunity in all the newborn and to reim munize any persons who may have lost their immunity to either virus

In 1945 an experiment was carried out under United Nations Relief and Rehabilitation Administration (UNRRA) auspices the purpose of which vas to demonstrate the efficacy of the scratch method of vaccination with ieurotropic yellow fever virus and to compare its immunizing potency with hat of the 17D vaccine Groups each comprising approximately 210 French military personnel none of whom had ever been outside France were vacci ated (*a*) by the scratch method employing neurotropic yellow fever virus lone (*b*) by the scratch method employing the same yellow fever virus plus accinia virus (*c*) by inoculation with 17D vaccine supplied in part from Wellcome Research Institution stock and in part from stock manufactured at the Rocky Mountain Laboratory of the United States Public Health Service

Code numbered blood specimens all taken approximately one month after

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mission of virus derived from inoculated persons was indicated by
work of Peltier, Durieux, et al. (1939*a*) who found that aegypti mosqu
fed upon persons vaccinated in this way with the neurotropic virus r
maintained the virus and even when they did were unable to transm
by bite. Other workers have obtained successful transmission of neurotro
virus to both monkeys and mice (Davis, Lloyd, and Frobisher, 1932) throu
the agency of aegypti, but such experiments may have involved virus havin
different pathogenic properties and certainly involved different hosts. In
they therefore do not necessarily invalidate the results of Peltier, Durieux
et al. (1939*a*).

Peltier, Durieux, et al. (1940) reported the first extensive field trial of
neurotropic virus applied topically, the results of which were satisfactory.
The work of the Dakar group was pursued throughout the years of the
Second World War under difficult circumstances and was given little public
ity until the cessation of hostilities. In a postwar publication Peltier (1947)
reported the vaccination of more than 14,000,000 persons by this method.
The program as presently conceived involves vaccination of the entire popu
lation of the colonies with the two viruses administered simultaneously once
every 4 years in order to insure immunity in all the newborn and to reim
munize any persons who may have lost their immunity to either virus.

In 1945 an experiment was carried out under United Nations Relief and
Rehabilitation Administration (UNRRA) auspices, the purpose of which
was to demonstrate the efficacy of the scratch method of vaccination with
neurotropic yellow fever virus and to compare its immunizing potency with
that of the 17D vaccine. Groups each comprising approximately 210 French
military personnel, none of whom had ever been outside France, were vacci
nated (*a*) by the scratch method employing neurotropic yellow fever virus
alone, (*b*) by the scratch method employing the same yellow fever virus plus
vaccinia virus, (*c*) by inoculation with 17D vaccine supplied in part from
Wellcome Research Institution stock and in part from stock manufactured
at the Rocky Mountain Laboratory of the United States Public Health
Service.

Code-numbered blood specimens, all taken approxim

the vaccinations and including as controls 30 samples from unvaccin persons were tested as unknowns in the laboratory of the Pasteur Insti at Dakar and in the Rocky Mountain Laboratory In the former laborat an intracerebral protection test method of considerable severity was e ployed In the latter the tests were done by the intraperitoneal techniqu Some discrepancies occurred in results obtained in the two laboratories an as many of the sera as were available in sufficient quantity were sent to the Yellow Fever Laboratory in Rio de Janeiro for further tests The results of the experiments were published in the Epidemiological Information Bulle tin 1916 They demonstrated conclusively that the Dakar vaccine and the scratch method of applying it are highly effective in inducing immunity to yellow fever In both groups receiving it the presence of neutralizing anti bodies was demonstrable in almost 100 per cent of individuals The apparent discrepancies in results with sera of persons receiving the 17D vaccine when these were tested in three different laboratories were almost certainly due to the use by the Rocky Mountain Laboratory of a protection test method that was more sensitive than the methods used in the other two laboratories However these discrepancies probably indicate that the response evoked by the Dakar vaccine resulted in the formation of more antibody per person than that evoked by the 17D vaccine

The time of appearance of the antibodies following vaccination by the scratch method and the duration of the induced immunity have not been as carefully studied as in the case of the 17D vaccine However from what is known of the antigenic properties of this and other strains of yellow fever virus it might be surmised that the response is prompt As to duration of he immunity Peltier (1917) reported the finding of 85 9 per cent of persons mmune after 4 years and 82 per cent after 7 years The test method used these studies was not mentioned but it seems probable that the figures are nservative and that the actual immunity rate was at least as high as stated

e technique of preparing and administering the vaccine by the scratch hod was described in detail by Peltier (1917) Reactions to the neuro ic virus applied by this method are probably more frequent and aps a little more severe (Peltier 1918) than those that ensue after lation with the 17D vaccine However, the reported reactions in West a and those in the UNRRA experiment were not such as to contra te use of the method

TOPICAL APPLICATION OF 17D ATTENUATED VIRUS

The potential hazards inherent in the use of mouse brain and of the mous adapted yellow fever virus and on the other hand the demonstrated effec tiveness of the Dakar method of immunization led to further studies seekin elimination of the potential dangers Hahn working in Nigeria inoculated separate groups of volunteers with the 17D and French neurotropic viruses by topical application Both viruses had been grown in chick embryos He made tests for circulating virus on members of each group from the 4th to the 7th day after the application of the vaccines Twenty three nonimmune persons received each vaccine Only one of the 23 receiving the 17D vaccine showed circulating virus this being on the 4th day By contrast 14 of the 23 (60 8 per cent) receiving the French virus exhibited that agent in their sera on one or more days The high incidence of circulating virus following topical application of the French virus afforded a relatively safe method of perform ing challenge inoculations in man to correspond to those made in monkeys with virulent pantropic virus Hahn then inoculated another group of 19 nonimmune persons by topical application of 17D virus and challenged them 4 days later with the French virus both viruses again being cultivated in chick embryos As controls he used a group of 17 nonimmunes inoculated with the French neurotropic virus alone Tests for circulating virus in the two groups were made on the 6th and the 8th day after the inoculation of the French neurotropic virus with the results shown in Table 6

These results indicate that the 17D virus applied topically in human beings induces sufficient protection within 10 days to prevent infection completely

A field trial was made at Kumba in the British Cameroons where approxi mately four thousand persons received topical applications of the chick em bryo 17D vaccine Postvaccination protection tests on previously nonim mune persons showed 93 per cent to have been immunized by the vaccine

The 17D vaccine employed by Hahn is prepared by grinding the whole virus infected chick embryos with added gum arabic and desiccating the mix ture At the time of use it is necessary only to rehydrate with saline or dis tilled water Additional experiments have been made combining vaccinia lymph with the 17D yellow fever vaccine pulp and desiccating both together Field trials with the latter have given very promising results Further investi gations on the topical application of 17D virus as a method of

against yellow fever are in progress It is possible that they may lead t
safest effective yet inexpensive vaccine against this disease

TABLE 6

RESULTS OF TESTS FOR CIRCULATING VIRUS IN PERSONS RECEIVING 17D VIRUS BY TOPICAL AP-
CATION BEFORE AN INOCULATION OF FRENCH NEUROTROPIC VIRUS AND IN CONTROLS RECEIV-
FRENCH NEUROTROPIC VIRUS ALONE

Group	Inoculation	Test for circulating virus			
		6th day after inoculation of French neurotropic virus		8th day after inoculation of French neurotropic virus	
		No tested	No positive	No tested	No positive
A (19 persons)	Topical application of 17D virus challenged 4 days later with French neurotropic virus	18	0	19	0
B (17 persons)	Inoculated with French neurotropic virus as controls	17	10	17	8

RELATIVE MERITS OF THE 17D AND DAKAR VACCINES

In the present state of knowledge certain points seem to be in favor of the Dakar method others seem to be against it The use of the neurotropic virus which is known to be more pathogenic for man rhesus monkey and mouse than is the 17D virus may be a potential hazard Even though encephalitis has not been prevalent among persons vaccinated by this method the possibility cannot be ignored that it may on occasion occur The use of mouse brain virus seems to be a more objectionable feature There is always the possibility that the yellow fever virus may become contaminated with an other virus that the mouse may be harboring—lymphocytic choriomeningitis for example—with resultant accidental infection in recipients of the vaccine Lastly there is also the potential hazard whenever mammalian nervous tissue is employed in a vaccine of allergic demyelinating encephalomyelitis

Two points strongly in favor of the Dakar method are its (...

its demonstrated antigenic effectiveness It is probable that the quantity antibody formed following vaccination with the mouse brain virus is great than that elicited by inoculation with the 17D chick embryo vaccine neve theless experience indicates that the immunity induced by 17D vaccine i adequate for protection The relatively low cost of immunizing by the Dakar method is a strong point in favor of any product which is to be ad ministered on a total population basis

Consideration of the foregoing facts suggests that the topical application by the scratch method of either the neuroadapted or the 17D virus cultivated in chick embryos may offer the optimal solution to all the problems involved

PASSIVE IMMUNITY

Each of the methods for the performance of yellow fever protection or virus neutralization tests is dependent on the passive transfer of antibody from one host to another The effectiveness of passive immunity in this disease as related to experimental procedures is therefore well established Its value for clinical use is however not so clearly demonstrable

Bauer (1931*b*) conducted experiments to determine the length of time during which passive immunity persists in rhesus monkeys He found that homologous serum given to rhesus monkeys in 2 0 cc quantities protected them fully for 3 and partially for 6 weeks against a dose of virus that more recently developed methods (Reed and Muench 1938) indicate was at least 1 000 LD_{50} In another experiment he injected monkeys with human serum in 5 0 cc doses challenged them with a dose of virus approximating 100 000 LD_{50} and found that those receiving serum 1 day or 7 days prior to challenge were protected while those receiving the serum 11 or more days before chal lenge were fully susceptible It is thus evident that both homologous and heterologous sera are effective in the passive transfer of immunity but the effect of heterologous serum may be more transient owing probably to its being inactivated sooner

In the early days of experimental work on yellow fever prior to the de velopment of effective methods for vaccinating against the disease accidental laboratory infections were so prevalent that in some laboratories it became routine practice to administer immune serum periodically to laboratory workers in order to protect them against accidental infection Moreover administration of immune serum was practiced in the event of a known lab oratory accident that it was thought might lead to the infection of

(Bauer and Hudson 1928b Burke and Davis 1930) The former proced
seemed to be of little value (Burke and Davis 1930) and the experim
of Bauer (1931b) would indicate that in order to be effective the inoculati
of serum would have to be given at a reasonably short interval before t
exposure occurred The immediate inoculation of immune serum followi
a laboratory accident may have been beneficial as N C Davis (1934b
showed the procedure to be effective up to 48 hours after inoculation o
monkeys and furthermore reported a very mild probably abortive attack
of yellow fever in a worker who received immune serum promptly follow
ing a laboratory accident Davis also showed and it has been the experience
of others before and after him that immune serum is without any beneficial
effect once the onset of the disease has taken place

HYPERIMMUNITY

Pettit and Stefanopoulo (1933) reported the production in horses of hyper
immune sera against yellow fever virus and a little later Hughes and Lloyd
(Lloyd 1935) produced in goats hyperimmune sera having 15 times the
protective power usually exhibited by the sera of human beings convalescent
from yellow fever Whitman (1935) made quantitative studies of the reac
tions of rabbits to single and repeated inoculations of yellow fever virus in
small and large doses He showed that the virus does not multiply in rabbits
and may be demonstrated in the circulation only within 24 hours of inocula
tion regardless of the dose given Quantitatively the antibody response
elicited was greater following large doses Furthermore he found that in
abbits immunized by one injection of viable virus and then given a rest
eriod a second injection of virus evokes a further marked but transitory
oduction of antibodies the titers at peak levels reaching values 10 or 20
nes those attained following the initial inoculation The transitory nature
the hyperimmune response was shown by the fact that the peak level of
ibody was demonstrable 14 to 21 days after reinjection and that the titers
eeks after reinjection were significantly below the maximum attained
heiler and Smith (1936) applied the methods of Whitman (1935) to a
of the antibody response in rhesus monkeys and observed a similar
immune response in these animals The peak levels of antibody in the
eys were attained between the 7th and the 11th day following reinocu
with a large dose of virus They therefore exsanguinated their monkeys
9th day after reinoculation quantitative studies with pooled 9

monkey serum showed antibody titers 10 to 20 times that of human immu
serum

The development of effective methods of immunization against yello
fever without the use of immune serum has obviated the need for the ser
that the foregoing experiments were undertaken to supply. Nevertheless the
studies made available methods that may be relied upon to produce high
titer antisera for whatever purpose these may be required.

APPLICATIONS OF IMMUNOLOGIC METHODS IN YELLOW FEVER INVESTIGATIONS

Inasmuch as the precipitating and complement binding antibodies quickly disappear following yellow fever and complement binding antibodies are not commonly formed after vaccination, the only immunologic procedure that has general application is the neutralization or protection test. The choice of method for the latter is somewhat a personal matter. Although the evidence is strong that the intraperitoneal test, in particular that employing immature mice, is more sensitive in the detection of antibody, either this or the intracerebral test may be depended upon to produce reliable results when sufficient attention is given to details. In the discussion that follows the applications of the yellow fever protection test are listed and certain laboratory and field procedures that have proved of value are set forth.

IMMUNITY SURVEYS

Owing to the widespread use of yellow fever vaccine and in view of the fact that it is impossible to distinguish by any protection test the immunity induced by vaccination from that which results from natural infection, it is now necessary to exercise considerable caution in the selection of human beings as blood donors for surveys of immunity undertaken with the purpose of defining the distribution of natural infection. However, the areas in which mass vaccination programs have been executed are a matter of record and are known to local health authorities. In other areas vaccine has been administered principally to travelers—who should be in possession of certificates—and to men who served in the armed forces during the war. Blood samples from indigenous adult females and children residing in areas where mass vaccination has not been done will therefore be relatively, if not completely, free of influence from vaccination, if travelers are

The statement of a man who may have served in the armed forces that he was or was not vaccinated against yellow fever cannot be accepted at face value

The knowledge acquired during the last 18 years concerning jungle or sylvan yellow fever points emphatically to the fact that the most important localities for immunity surveys in man are inhabited areas situated in close



Fig. 26 Collecting blood specimens for immunity surveys in Uganda, East Africa

proximity to permanent forest It is these localities and the forest itself from whence stem the infections that give rise to rural and urban epidemics of the disease Moreover, the accumulated evidence indicates that the animals within the forest, especially the wild primates, may yield as much information relative to the incidence of the disease as does the human population, or perhaps more

The collection of blood samples from human beings or from certain wild animals for protection tests is best accomplished by means of vacuum venules, most of which are stronger than ampules and less likely to break in transit (Figs 26 and 27) Skill in the use of venules is a matter of practice and is easily acquired Separation of serum from the clots in the field is unneces

sary if the specimens can be protected from excessive heat and can be dispatched to the testing laboratory within a few days. Specimens are much more likely to become contaminated when separation of serum is done in the field than when it is carried out under the more suitable conditions of the laboratory. Should it be deemed imperative to separate the serum in the field this may be done by inserting the needle of an empty venule through



Fig 27 The use of vacuum venules for the collection of blood samples in the field for yellow fever control at Aguas Claras, Bayano River, Panama

the iodized stopper of the filled one and releasing the vacuum of the former. In the case of the smaller species of wild animals blood specimens may be more easily obtainable with syringes than with venules. By either method it is advisable in most of the smaller animals to open the thorax widely before any attempt is made to collect blood.

Kerr states that hemolysis of serum may be highly objectionable if Friedländer's bacillus or enterococcal infection is present in the mice used for the tests. Hemoglobin promotes the growth of these organisms and gives rise to nonspecific deaths of mice.

The manner of handling specimens may have an important effect on the results. In collecting blood from human beings the collector should be assisted by a clerk who makes accurate records of the name, age, sex

place of domicile of each donor The collector and the clerk or
should both hold themselves responsible for insuring that the info
on the label of each specimen corresponds to that in the record th
(usually a numbered piece of adhesive tape) should be affixed to th
men by the collector immediately the sample is taken When the ve
filled and the label is attached the needle should be removed flush
the rubber stopper and the butt sealed with melted wax In case the va
has not been exhausted as is often the case failure to exercise this precau
may result in bacterially contaminated clot being sucked into the venule
any subsequent time slight pressure is brought against the needle Th
venules should be packed in a compartmented container in the erect po
tion this prevents the exposure of a large surface of clot to the washi
action of the overlying serum whenever the container is moved and th
tends to minimize hemolysis and breaking up of the clots Toward the sam
end all unnecessary shaking or jostling of samples is to be avoided A copy
of the sampling record should be packed in the container of specimens for
shipment to the laboratory Promptness of dispatch and of handling in the
laboratory serve to minimize hemolysis

The collection of specimens from wild animals may be done after trapping or the animals may be taken with firearms In the latter case it is advisable that the collector be equipped with a vacuum flask and ice for the refrigeration of potentially or actually contaminated specimens and that such specimens be kept chilled until they can be filtered prior to testing The presence of a few bacteria in a specimen does not necessarily render it valueless if bacterial growth can be prevented

Protection tests may be of very great value in detecting the presence of yellow fever or in determining its incidence through resurveys of individuals known to have been previously nonimmune who can be identified with certainty Identification may be accomplished in such instances by the finger print method Mahaffy Smithburn et al (1942) employed this method in a resurvey of finger printed nonimmunes and discovered an outbreak of the disease of considerable proportions that might otherwise have escaped notice Recapture and retesting of previously nonimmune wild animals might be expected also to yield valuable information on the occurrence of yellow fever This method has been employed in Brazil but the only recorded use was in an area where yellow fever was anticipated but did not occur (Laemmert Hughes and Causey 1949)

Routine methods may be employed in the testing of human sera and a

protective action of the serum may be taken as very nearly absolute evidence that the individual (if over 6 months of age and therefore not immune by inheritance) has been infected with yellow fever virus either in nature or by vaccination. This is equally true for most wild primates. Modified methods may be required for certain animal species, as Bugher (1940) has shown, in that dependable results may require the use of larger doses of virus in the test than is usual. Protection tests with the sera of certain species, notably birds, cattle and sheep, are not to be relied upon in the present state of knowledge.

PROTECTION TESTS IN DIAGNOSIS

Under ideal circumstances, protection tests may be equivalent diagnostically to the isolation of virus from the individual. In order to establish the diagnosis in this way, two specimens of blood must be tested. The first must be obtained prior to the development of antibody, and the second must be taken long enough after the onset of disease so that antibody is demonstrable. The diagnosis of yellow fever is established if the first specimen is negative and the second protective, or if the second specimen contains a significantly greater amount of antibody than the first. No other result may be accepted as proof that the disease in question was yellow fever. A strongly protective specimen obtained very early in the disease is firm evidence that the illness was not yellow fever.

IMMUNOLOGIC METHODS IN STUDIES ON MAMMALIAN HOSTS

The knowledge that is most important in determining the susceptibility of mammalian hosts to yellow fever virus and their potential epidemiologic importance is (a) whether they can be infected by arthropod vectors, (b) whether they circulate virus in sufficient quantity to infect other vector insects and thus act as a temporary reservoir host, and (c) whether they develop demonstrable antibody following the bites of infected mosquitoes or the inoculation of a small dose of virus.

No animal has yet been found that exhibits circulating virus following the bites of infected vector insects or following inoculation of a small dose of yellow fever virus without, as a consequence of this, developing neutralizing antibodies. Therefore, if a given species of animal is found in nature in possession of specific neutralizing antibody, it may be accepted that the species concerned can be infected by vector insects. Special methods of

testing sera of some species may be required as Bugher (1940) and W (1943) have shown in order to be certain that the neutralization of is brought about by specific antibody No standard protection test m may therefore be employed as a routine for the testing of all sera from ever animals they may come When the tests are made sufficiently s to overcome nonspecific neutralizing factors yet sensitive enough to d antibody in small quantity surveys of immunity to yellow fever in wild mals may be expected to yield informative results

The reaction of a given species of animal to infection may be determin either by allowing the animal to be bitten by infected insects of know vector potential or by inoculation by syringe The susceptible host respond to such experimental infection by allowing the virus to multiply in it tissues and if it survives by developing specific antibody In most animal species the resultant antibody persists and the animals remain immune How ever Anderson and Roca García (1947) have shown that at least one species of animal may be infected experimentally produce antibody lose its im munity and again become infectible Whether animals exhibiting such be havior have sufficient virus in their blood at any time to infect vector insects is not known

The progression of antibody formation may be demonstrated in suscep tible species if protection tests are done at daily intervals from the 4th day onward following inoculation It is not uncommon with such procedure to observe that the sera taken on successive days will give first negative then inconclusive and finally protective results (Smithburn and Haddow 1949)

PROTECTION TESTS FOR IDENTIFICATION OF VIRUS

Although in many instances other evidence is sufficient to establish with reasonable certainty the identity of a newly isolated virus recourse is in variably had to immunologic methods for final identification If all the evidence for identification is not in complete agreement it is usually not the immunologic findings that are found to be faulty Until a specific immune serum can be prepared for a newly isolated virus the only immunologic method available for its identification is to test it against antisera that investigators suspect may neutralize the agent In the case of many freshly isolated strains of yellow fever virus the pathogenicity for mice by intra peritoneal inoculation even after the intracerebral inoculation of starch solution may be so irregular or so low that the usual methods employed in

the intraperitoneal test cannot be relied upon. Under such circumstances a satisfactory test may be made by mixing several decimal dilutions of virus with equal portions of normal and immune serum, incubating the mixtures and inoculating each intracerebrally into six or more susceptible mice. If the titer of virus is 2 or more decimal dilutions higher in the normal serum than in the yellow fever immune serum, this may be taken as very strong evidence that the virus is that of yellow fever. Final and unequivocal evidence may be obtained by producing an antiserum against the newly isolated agent and testing this against yellow fever virus. Reciprocal cross neutralization of the order of 100 or more LD_{50}, with the serum that neutralizes one virus most strongly behaving similarly toward the other, may be taken as final evidence of identity of the two.

Similar methods are usually effectual not only in identifying a newly isolated strain of yellow fever virus (Mahaffy, Hughes, et al., 1941) but also in determining that a given virus is not that of yellow fever and showing that it is another known virus (Smithburn, Haddow, and Gillett, 1948; Dick, 1949; Warren, Smadel, and Russ, 1949), or in establishing that the agent is immunologically different from previously known viruses (Smithburn, Hughes, et al., 1940; Smithburn, Mahaffy, and Paul, 1941; Smithburn, Mahaffy, and Haddow, 1944; Roca-García, 1944; Smithburn, Haddow, and Mahaffy, 1946; Laemmert and Hughes, 1947).

CHALLENGE INOCULATION

Challenge inoculation has proved a very useful method in studies of the effects of vaccination and for other purposes as well. Fox (1943) applied the method to a study of mice that showed no clinical reaction and to others that sickened and recovered following inoculation with the 17D attenuated yellow fever virus. He was able to show that inapparent and sublethal infections occur following administration of the 17D virus and that these are associated with the development of specific antibody and of resistance to *reinoculation*. Fox emphasized, *however*, that such *sublethal and inapparent* infections are rare following inoculation with pantropic or neuroadapted virus. The latter fact, together with the well-recognized presence of occasional resistant individuals in most lines of mice, would seem to limit the usefulness of challenge inoculation of mice as a method of detecting the presence of yellow fever virus in materials from the field.

5 THE ARTHROPOD VECTORS OF YELLOW FEVER

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In reviewing the development of our knowledge of the relationship between arthropods and the spread of yellow fever, one cannot but be impressed by the extraordinary contribution of Walter Reed and his collaborators (United States 61st Congress, Yellow Fever, 1911). Working at a time (1900–1901) when little was known of viruses as disease agents, when there was but meager evidence pointing to the possibility of insect transmission of human disease, and when the only experimental animal was the human volunteer, these men were able to establish certain basic facts regarding yellow fever that have remained virtually unchallenged during the last 50 years. They pointed out that while the disease is noncontagious, the causative agent is to be found in the blood stream, although for only a few days following the onset of illness. They showed that this agent is filtrable, that when it is ingested by a mosquito of a particular species (*Aedes aegypti*—then known as *Culex fasciatus* and later as *Stegomyia fasciata*) (Fig. 28) it remains alive in the mosquito for a period approaching the life span of the insect, but that the mosquito cannot transmit this agent to a susceptible person until approximately twelve days after the infecting meal. Thus they demonstrated that the mosquito is the reservoir of the infectious agent, and that the vertebrate host is of only transitory importance in the maintenance of the virus.

The large mass of evidence gathered later by using experimental test animals has tended to reinforce rather than to modify these observations. The discovery that yellow fever is not restricted to human beings but can exist in the forest as a disease of forest vertebrates has extended the epidemiologic concept, so that investigators are no longer concerned with the single vertebrate, man, and the single mosquito, *A. aegypti*. However, the basic concept as established by the Reed commission of a vertebrate-mosquito cycle with the mosquito acting as the reservoir has as yet not been displaced. As far as is known, this is still the sole method by which the virus is maintained in nature.

Although other workers, in particular Marchoux, Salimbeni, and Simond (1903), confirmed the Reed commission's findings, the necessity for using human volunteers so restricted experimentation that no significant advance in knowledge of the insect transmission of yellow fever was possible during the next 26 years. With the discovery of Stokes, Bauer, and Hudson (1928*a*)

that the rhesus monkey was susceptible to the virus of yellow fever, a
of investigation was made possible.

The first laboratory studies not only confirmed and extended the o
tions of the Reed commission with reference to *A. aegypti* but showe
other mosquitoes were experimentally capable of transmitting the d
Bauer (1928) Philip (1929*a*, 1930*a*), Kerr (1932), and other workers
onstrated that *Aedes* of several species, as well as members of the ge

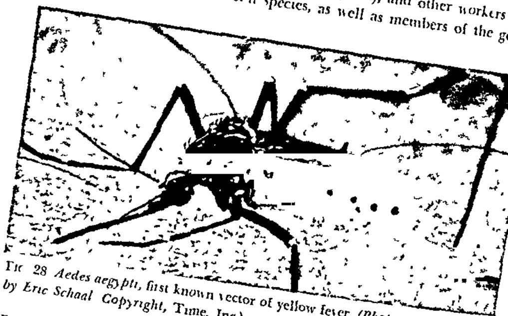


Fig 28 *Aedes aegypti*, first known vector of yellow fever (*Photographed for Life by Eric Schaal Copyright*, Time, Inc.)

Eretmapodites, *Taeniorhynchus* (then known as *Mansonia*),[1] and *Culex*, could transmit yellow fever in the laboratory and therefore could potentially serve as vectors of the disease in Africa During this period Davis and Shannon (1929*c*, 1931*a* and *b*) found that several species of South American *Aedes* were experimentally capable of a similar role and, furthermore (1929*b*) that a Brazilian strain of yellow fever virus as well as the African Asibi strain could be transmitted by *A aegypti* Many of these observations were somewhat academic in nature at the time inasmuch as yellow fever was still considered to be a disease of man alone and therefore transmissible only by a mosquito primarily associated with man In fact, so strong was this belief that the validity of reports of cases of yellow fever in Muzo, Colombia

[1] There is still confusion concerning the correct nomenclature for this genus Under the International Rules of Zoological Nomenclature *Taeniorhynchus* falls as a synonym for *Aedes* However the British have accepted *Taeniorhynchus* as a replacement for *Mansonia* and many other entomologists have followed suit

received on several occasions was seriously doubted (Gorgas 1917 and others) because of the absence of *A. aegypti* in that locality. This same conviction was probably responsible for the lack of attention paid to the provocative and prophetic report by Franco, Martínez Santamaría and Toro-Villa (1911) who pointed out that yellow fever in Muzo was contracted during the day while the men were at work and not around the dwellings, and suggested that forest mosquitoes present there might perhaps be responsible for the transmission.

During this period much basic research was done on the behavior of yellow fever virus in *A. aegypti*. In particular, studies by N. C. Davis (1932a) showed that the extrinsic incubation period—the term coined by Carter (1900) to cover the period from the mosquito's infecting meal to the time when it could transmit the virus—was related to temperature. Various authors contributed to the knowledge that virus transmission persisted throughout the life span of the mosquito and that the amount of virus in the mosquito, despite an early fall shortly after feeding, subsequently reached and remained at a high level.

In 1932 an outbreak of undoubted yellow fever occurred in the Valle do Chanaan in Espirito Santo, Brazil, in the virtual absence of *A. aegypti*. This outbreak, described by Soper, Penna, et al. (1933) conclusively destroyed the notion that in nature yellow fever is restricted to man and *A. aegypti* and forced the rapid recognition of jungle yellow fever as a basic epidemiologic concept. The previous studies on mosquitoes other than *A. aegypti* were now of direct interest, and the emphasis turned to the investigation of forest arthropods (Figs. 29 and 30). This approach was rapidly given impetus in South America when Shannon, Whitman, and Franca (1938) found wild-caught mosquitoes of the genus *Haemagogus* as well as *Aedes leucocelaenus* infected with the virus of yellow fever and capable of transmitting it by bite to susceptible laboratory animals. In particular, the members of the genus *Haemagogus* were of interest in that their predilection for feeding during the noon hours increased the danger to humans entering the forest in the course of routine activities. Certain lacunae in the knowledge of the seasonal distribution of these mosquitoes existed until an observation in Colombia (Bugher, Boshell-Manrique, et al. 1944) showed that their prevalence was not accurately determined by routine ground captures, and that they were primarily residents of the forest canopy. A study of their vertical distribution was therefore essential in order to explain their relationship to susceptible vertebrate hosts.

While these investigations were being conducted in South America investigations of a like nature were undertaken in Africa for the purpose of learning whether similar mosquito habits could explain the maintenance and transmission of the disease on that continent Results of these studies especially in East Africa, indicate two cycles of transmission a forest mosquito—forest vertebrate cycle (Smithburn, Haddow, and Lumsden 1949)



Fig 29 The Brazilian yellow fever inspection service covers rural areas in the Amazon Valley in a search for mosquito larvae (1943)



Fig 30 Mosquitoes caught at the capture station at Passos, Minas Gerais Brazil are preserved alive in shell vials

and a man—peridomestic mosquito cycle, similar to the man—*A aegypti* cycle (Mahaffy Smithburn et al, 1942) In the forest cycle, *Aedes africanus* has been shown to be the chief vector, and in the peridomestic cycle, *Aedes simpsoni*

This is the current status of knowledge concerning the spread of yellow fever there are mosquito vectors of known efficiency and of known distribution These have been found associated with susceptible vertebrate hosts and yellow fever virus has been isolated from specimens caught in nature during times when the disease was proved to exist either in man or in other vertebrates The evidence is all in favor of mosquitoes being the agents of transmission

ROLE OF MOSQUITOES IN THE TRANSMISSION OF YELLOW FEVER

Studies of arthropods in relation to yellow fever have been largely confined to mosquitoes This is due in part to the success of these investigations and in part to the fact that preliminary studies with other groups of insects have not yielded sufficient positive evidence to stimulate further inquiry into their possible relationship to the transmission of the disease in nature Because of this the sections following will be restricted to studies on mosquitoes only What little knowledge there is on the response of other arthropods to yellow fever virus will be summarized at the end of this chapter

Two factors are of cardinal importance in determining the capacity of a mosquito to transmit yellow fever The first is the fate of the virus ingested by the mosquito the second is the degree of contact between the mosquito and susceptible vertebrates It is therefore advisable to summarize present knowledge of these two factors in a general way before discussing current beliefs concerning the specific mosquito vectors in South America and Africa

RELATIONSHIP BETWEEN MOSQUITOES AND YELLOW FEVER VIRUS

SUSCEPTIBLE MOSQUITOES

To transmit yellow fever a mosquito must serve as a suitable habitat for the virus If the virus rapidly disappears from the mosquito s body transmission becomes impossible To be sure there remains the chance that a nonsusceptible mosquito might be interrupted while feeding on an infected vertebrate with virus in its blood and that the insect with its proboscis coated with virus might fly to another vertebrate and mechanically infect this animal However in laboratory studies on mechanical transmission of yellow fever by mosquitoes Philip (1930*d*) and Kumm (1932) failed to infect experimental animals in this way *The probability that such transfer* of virus can occur under natural conditions with sufficient frequency to maintain the cycle of transmission is virtually nil In general therefore no vector importance can be attached to those species of mosquitoes that may be considered nonsusceptible namely those in which the virus cannot become established following an infecting blood meal Unfortunately the rea

sons for this insusceptibility to yellow fever virus have not been explored. In this connection it is interesting to note that Merrill and Ten Broeck (19 in studies on eastern equine encephalomyelitis were able to convert spe mens of nonsusceptible *A. aegypti* into susceptibles which transmitted t virus by bite by puncturing the blood-filled stomachs of the recently en gorged females. This they believed implied that the nonsusceptibility wa due to the failure of the virus to penetrate the gut wall. Once this barrier was mechanically broken the virus was capable of establishment in the mosquito. They speculated on the possibility that bacterial or other parasitic mosquito infections might promote an equivalent mechanical injury under natural conditions. However, since W. A. Davis (1940) was able to transmit the same virus with untreated specimens of *A. aegypti*, it is probable that the findings of Merrill and Ten Broeck were applicable only to a minor colony variant. Whether some of the mosquitoes considered to be nonsusceptible to yellow fever could be similarly converted into susceptibles is not known. The possibility remains that parasitic infections of mosquitoes occurring in some geographic areas and not in others might produce localized susceptibles in a normally nonsusceptible species.

Turning now to the mosquitoes susceptible to infection with the virus of yellow fever, it has been noted repeatedly that there are many species that may harbor this virus for long periods of time but are nevertheless unable to transmit yellow fever by bite. In this group the virus is obviously capable of penetrating the gut wall and establishing itself in the bodies of the mos quitoes. Whitman found that the amount of virus recoverable from the bodies of *Taeniorhynchus fasciolata* (then known as *Mansonia fasciolata*) was as great as or greater than that recoverable from *A. aegypti* fed at the ame time on the same monkeys. Yet *T. fasciolata* failed to transmit the rus by bite. The explanation of this phenomenon is lacking. Whether the rus is incapable of entering the salivary gland or whether it does penetrate t is neutralized by virucidal substances in the saliva is not known. Whether the group of susceptible nontransmitters is potentially important he dissemination of yellow fever is debatable. Bauer and Hudson (1928*b*) ed that yellow fever virus could readily pass through the unbroken of experimental monkeys, yet they failed to transmit yellow fever by ng infectious mosquito emulsions on the unbroken skin. Aragão and osta Lima (1929*a*), on the other hand, claimed to have infected ys by merely dropping mosquito dejecta on their unbroken skin. an and Antunes (1937) in their studies on *T. fasciolata*, twice failed

to transmit the disease to monkeys by crushing the bodies of mosquitoe this species on the skin and scratching the debris with the finger nails. Fr the limited information available therefore it seems unlikely that virus c be transmitted in nature to a significant extent by such mosquitoes. How ever, as will be discussed in more detail later, it must be borne in mind th the majority of the studies made on this group have been done with on strain of virus (Asibi). Whether some of these species could transmit other strains of virus is not known.

Before dismissing this group it is worth noting that N. C. Davis (reported by Whitman and Antunes, 1937) was able to transmit yellow fever by the bite of one lot of *Psorophora ferox* though he had failed to do so in earlier experiments with the same species (Davis and Shannon, 1931b). Whitman and Antunes (1937) in further transmission experiments with mosquitoes of this species failed to get transmission by bite. This discrepancy in results suggests that variation may exist within species, a possibility which has never been adequately explored.

Basically the interest of the epidemiologist lies in those mosquitoes which, aside from being susceptible, are also capable of transmitting yellow fever virus by bite, and studies have been devoted chiefly to those species. At this point the various factors that have been shown experimentally to influence the relationship between the mosquito and the virus will be discussed briefly.

DISTRIBUTION OF VIRUS IN AN INFECTED MOSQUITO

One of the foremost problems to be attacked was the question of what happens to the virus of yellow fever in the mosquito. Bauer and Hudson (1928a) were the first to show that although mosquitoes of the species *A. aegypti* were unable to transmit yellow fever by bite for a period of time fol lowing their infecting meal, the virus was nevertheless demonstrable throughout this period if the mosquitoes were ground up and injected into susceptible rhesus monkeys. The extrinsic incubation period thus repre sents the time requisite for the virus to get from the stomach of the mos quito to its salivary glands. The authors were able to establish this incuba tion period at 9 to 12 days at the temperatures encountered (71-90°F.).

Hindle (1929), Aragão and da Costa Lima (1929b), and Davis and Shannon (1930) demonstrated that the virus of yellow fever was widely disseminated the bodies of *A. aegypti*. Hindle, for example, showed that the

tents and abdomens of infected aegypti contained virus. He failed to demonstrate virus in the legs. However, Aragão and da Costa Lima found an emulsion of legs to be infectious and the hemocelic fluid as well. Davis and Shannon went further in their dissections. Not only did they find the virus present in an emulsion of legs and wings, but they also demonstrated it in the ovaries, the midguts, and the hindguts. Surprisingly they failed to demonstrate it in the hemocelic fluid and in the hindgut contents. It should be noted that at the time these experiments were performed it was not yet known that yellow fever virus rapidly dies when suspended in physiologic salt solution in the absence of a minimal amount of protein. Thus the time between the preparation of the suspensions and their inoculation would have bearing on whether or not virus was to be recovered experimentally. Furthermore, suspensions of mosquito thoraces or abdomens would have more protein to protect the virus than would the content of the hindgut. From the results of these studies, then, it must be concluded that the virus is generally distributed throughout the body of the mosquito.

RELATION OF TEMPERATURE TO EXTRINSIC INCUBATION PERIOD

With regard to the effect of temperature on the length of the incubation period of yellow fever virus in mosquitoes, Marchoux and Simond (1906a) found that at 20°C *A. aegypti* failed to transmit by bite and that at 22°C the incubation period was at least 3 weeks or longer. Hindle (1930) showed that at 10 to 18°C virus could persist in the body of mosquitoes for long periods of time but without their being able to transmit it. When these mosquitoes were placed at 28°C for a week, transmission occurred. N. C. Davis (1932b) not only confirmed these observations but was able to establish a curve for the relationship between temperature and incubation period. At a temperature of 18°C, specimens of *A. aegypti* could be held for as long as 30 days without transmitting by bite, but positive results were obtained if they were then placed at a higher temperature for a few days. Even when stored for 4 weeks at as low a temperature as 8°C, they retained the virus, and when transferred to a temperature of 36°C for 6 days they transmitted it. At the other end of the scale Davis noted incubation periods as short as 4 days at 37°C, 5 days at 36°C, and 6 days at 31°C. At an average of 25.1°C the incubation period was 8 days and at 23.4°C it was 11 days. This relationship between temperature and incubation period implied that the virus was ac-

tively multiplying as previously suggested by Gay and Sellards (1927). However, in direct experiments Davis, Frobisher and Lloyd (1933) concluded that no true multiplication occurred largely because the virus recoverable at the end of the incubation period was always less than that recovered immediately after the mosquitoes had engorged although following a diminution in virus titer during the first week after feeding there was a secondary rise. Whitman (1937) suggested that the failure to find more virus in the mosquito after a suitable incubation period was due to the excessive amounts of virus in the stomachs of the freshly fed insects. By infecting his mosquitoes at a time when there was less virus in the freshly engorged blood he was able to recover more virus from the mosquitoes after an appropriate incubation period than they had initially contained. Whitman also found that the virus content of mosquitoes dropped rapidly during the first few days after an infective feeding and then started to increase again at about 7 days. In the experiments of Davis, Frobisher and Lloyd and of Whitman on virus multiplication temperature was not controlled. Subsequently Shannon and Whitman observed that the extrinsic incubation period was much longer in *A. aegypti* held at a constant temperature of 25°C than in specimens held at an average [illegible]°C, the rate of virus multiplication at temperatures in excess of [illegible]°C more than compensating for the slower multiplication at lower temperatures.

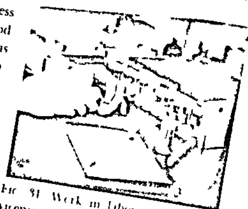


FIG. 31. Work in laboratory at Villavicencio, Colombia, where *Haemagogus* mosquitoes infected with yellow fever were studied.

This problem was studied in detail by Bates and Roca-Garcia who used *Haemagogus spegazzinii falco* instead of *A. aegypti* and two strains of yellow fever virus recently isolated from human yellow fever patients in Colombia (Fig. 31). In reporting their first series of studies (Bates and Roca-Garcia 1945) they presented figures to show that the extrinsic incubation period in *H. spegazzinii falco* (in their paper called *Haemagogus capricornii*) is markedly affected by temperature. In mosquitoes held at room temperature (21 to 27°C.) no transmission by bite was obtained at 10, 11, 1[illegible], and 20 days, the first positive result occurring after 22 days. In contrast, at

30°C transmitted by bite at 13 days and perhaps even at 10 days Unexpec edly these authors found that temperature not only affected the speed wit which the mosquitoes could transmit the virus but also the percentage o mosquitoes infected In brief studies on the above mentioned lots of mo quitoes they found by injecting ground up individual mosquitoes int susceptible baby mice that only tw of 19 specimens held at 24 to 27°C were infected while 13 of 19 speci mens held at 30°C were infected They repeated the studies using con trolled temperatures One group o mosquitoes was held at 20°C O these none of 10 examined con tained virus whereas 12 of 20 held at 30°C were infected In an inter mediate group held at 20°C for 16 hours daily and at 30°C for 8 hours 4 of 13 specimens contained virus In later studies (Bates and Roca Garcia 1946*b*) the authors con firmed and extended these observa tions Their first interest was in in vestigating the effect of temperature on the amount of virus recoverable from *H spegazzinii falco* at various time intervals following the initial

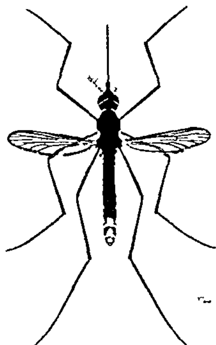


FIG 32 *Haemagogus spegazzinii*

virus ingestion (Fig 32) In mosquitoes held at 30°C the average virus con tent fell for the first 2 days then became stabilized and began to increase slowly In those placed at 20°C virus loss continued until the 5th day at which time it became stabilized When the latter mosquitoes were trans ferred after 10 days to a temperature of 30°C the virus titer of those in fected increased Mosquitoes of this lot (10 days at 20°C 6 days at 30°C) first transmitted virus by bite on the 16th day whereas the lot held through out at 30°C transmitted on the 13th day In mosquitoes kept at an interme diate temperature of 25°C virus was at a minimal concentration on the 4th day with subsequent increase With mosquitoes held for 20 hours daily at 25°C and 4 hours daily at 35°C results equivalent to a constant 30°C en vironment were obtained

In studies on the effect of temperature on the extrinsic incubation period the authors found that at a constant temperature of 25° C. transmission first occurred on the 28th day. With mosquitoes held 20 hours at 25°C. and 4 hours at 30° C. daily, it occurred for the first time on the 23d day. When a 4-hour daily exposure to 35°C. instead of 30° C. was introduced, the incubation period was 12 days. With a constant 24 hour exposure to 30° C. the incubation time was 10 days. However, in a further experiment their results indicated that these incubation periods are not uniform for all the mosquitoes in each group. Starting with a large group of the mosquitoes that had been fed on an experimentally infected monkey, they allowed individual mosquitoes to feed on individual baby mice at varying times following the infecting blood meal. The mosquitoes were held at 25° C. for 20 hours and at 35°C. for 4 hours daily. Between the 11th and the 15th day, 20 mosquitoes fed, and three (15 per cent) of them transmitted virus. Between the 16th and the 20th day two of 17 (13 per cent) transmitted virus. In subsequent 5-day periods, 58 per cent, 55 per cent, and 83 per cent of the mosquitoes tested transmitted. Between the 36th and the 60th day 11 of 12 mosquitoes (92 per cent) tested transmitted. This indicates that wide variations in the extrinsic incubation period exist between individual specimens, many not becoming capable of transmission for much longer periods of time than is indicated by the findings for the group as a whole.

It is of interest to note that these incubation periods, relative to temperature, are longer than the ones reported by Davis (*1932a*) for *A. aegypti* infected with Asibi virus. This could be taken to imply either that the species of *Haemagogus* used was a less effective vector than various species of *Aedes*, or that the strain of virus employed was responsible for the difference. However, as will be discussed more fully later, the explanation may be that as yet it has not been possible to match in the laboratory the truly optimal conditions for the development of the virus in mosquitoes of the *Haemagogus* genus.

Bates and Roca-García (*1946b*) called attention to several interesting details. In the first place they noted marked shortening of the extrinsic incubation period when their mosquitoes were held 4 hours daily at 35° C. instead of 4 hours at 30° C.—a shrinkage from 23 to 12 days. They pointed out that the temperature environment of 20 hours at 25° C. and 4 hours at 35° C. seems to be very favorable to the particular species of *Haemagogus* with which they were working, and noted not only that longevity is greater in this environment as opposed to a constant 30° C. temperature, but also that the

percentage of females laying eggs and the number of eggs per female were also greater In contrast to the apparently favorable influence of such a variable temperature environment on *H spegazzinii falco*, Bates (1947) in a more complete study of the effect of temperature on the development and longevity of mosquitoes noted that different species have quite different laboratory optima For example such mosquitoes of the forest floor as *Aedes serratus* and *P ferox* survived well in a subterranean room of high humidity and a constant temperature of about 25°C In this environment *H spegazzinii falco* did poorly In contrast when specimens of *A serratus* were kept under the conditions that doubled the mean life of *H spegazzinii falco* their own mean life fell to less than ⅓ of that demonstrated in the cellar environment

Perhaps this question of optimal environmental conditions has not been stressed sufficiently In all the early work on yellow fever virus in mosquitoes *A aegypti* has been the model Inasmuch as this species is already adapted to house life the laboratory environment is not far from a normal environment and could conceivably be an improvement upon it With the physiology of the insect in a healthy state its tissues may perhaps offer the best possible conditions for virus development As the mosquito is placed in a progressively less favorable environment it is quite possible that it becomes relatively less suitable as a host for the virus Furthermore the environment might include aside from temperature and humidity such additional factors as sunlight and the amount of flight in the cage As Bates and Roca García (1946b) pointed out it is possible that a mosquito *in flight* particularly in sunlight may develop a considerably higher internal temperature than the measurement of the surrounding air temperature would indicate Should this be so the degree of cage flight would have considerable bearing on the extrinsic incubation period Until more is known about the optimal environment for the species of mosquito being studied attempts such as those of Waddell and Taylor (1947) and Waddell (1949) to judge the relative vector efficiency of mosquitoes maintained under present laboratory conditions would seem to have a limited significance Although the data presented may represent a true picture of the relative efficiency of certain mosquitoes in the laboratory they may be quite inaccurate in representing the relative efficiency of these mosquitoes in their natural environment

Before leaving the question of the effect of temperature on the development of the virus in mosquitoes and its resultant effect on transmission there is one point worth speculating upon will a mosquito that is already

capable of transmitting by bite lose the ability to transmit yet retain virus if exposed to suboptimal temperatures On this point there is little reliable information Blanc and Caminopetros (1930) in studies on the transmission of dengue virus by *A aegypti*, presented evidence to show that mosquitoes that had already been able to transmit the virus by bite lost this ability when held at 16°C but recovered it when returned to an environment of 22 5°C In contrast Hindle (1930) infected 10 specimens of *A aegypti* with the virus of yellow fever and held them for 12 days at 28°C then for 57 days at a temperature of from 10 to 15 C At this time the bite of two of the mosquitoes produced the disease in a rhesus monkey Unfortunately no further experiments of this nature have been done with the virus of yellow fever despite the importance of such work from the standpoint of the transmission of the disease in nature Should virus levels in mosquitoes fall with lowered temperatures and transmission cease even though the number of living and infected mosquitoes remains constant it would make more difficult the recovery of virus from wild-caught mosquitoes at certain seasons particularly in biting experiments Furthermore if this did occur it would give additional support to the theory first suggested by Bates and Roca Garcia (1946*b*) that transmission is related to the general level of virus in the mosquito and is not a question of infecting the cells of the salivary glands

EFFECT OF HUMIDITY ON VIRUS DEVELOPMENT IN THE MOSQUITO

As was shown by Beeuwkes Kerr et al (1933) the life span of caged specimens of *A aegypti* is influenced by relative humidity In studies on the survival of these mosquitoes in Gadau in northern Nigeria they found that with either high or low temperatures low relative humidity shortened the average life span of the insects When they put their mosquitoes in a moist chamber the mean life was significantly increased Presumably humidity also has a bearing on the virus mosquito relationship in that it will influence the internal temperature of the mosquito both directly in affecting heat loss and indirectly by influencing the mosquitoes choice of microclimate However there are no data to indicate how significant such changes in relative humidity actually are with regard to the development of the virus

CAPACITY OF DIFFERENT VIRUS STRAINS TO DEVELOP IN THE MOSQUITO

In the foregoing summary of the effect of temperature on the development of the virus in mosquitoes, it was mentioned that *A. aegypti* and *H. spegazzinii falco* have apparently different rates of virus development at any one temperature. Aside from questions of physiologic optima, this difference could possibly be influenced by the strain of virus. Davis was using Asibi virus, while Bates and Roca García were using two native Colombian strains. Unfortunately, not much comparative work has been done with recently isolated field strains and Asibi virus in the same species of mosquito. Whitman and Antunes (1937) believed that *A. aegypti* took longer to transmit the M.A.J. strain than the Asibi strain, although no direct comparison was made. Inasmuch as a relationship has been demonstrated between vertebrates and virus strains (Laemmert, 1944), the question arises as to whether a similar relationship between mosquitoes and strains may exist. Waddell and Taylor (1948) offered provocative evidence. In attempting to pass yellow fever virus in series through marsupials of several species by means of *A. aegypti*, these authors had great difficulty in initiating their series. Using a Brazilian virus that had been maintained by syringe transfer for seven passages in rhesus monkeys and one passage in marmosets following its isolation from man, they were able to infect a marsupial (*Metachirus nudicaudatus*) by the bite of mosquitoes but were unable to infect normal mosquitoes by feeding them on the marsupial. They then made five passages of this virus, using mosquitoes as vectors and marmosets as hosts, but again failed to establish the virus in series in *M. nudicaudatus*. A 6th mosquito-marmoset passage failed to alter the situation. Following 11 mosquito–marmoset cycles, however, a series of transfers of virus from mosquito to *M. nudicaudatus* and back to mosquito was carried through eight cycles. Subsequently, using another recently isolated strain of Brazilian virus that had been through only seven mosquito–marmoset cycles, six mosquito–*M. nudicaudatus* cycles were completed without a failure to infect and transmit. The question presenting itself here is whether the apparent favorable change was due to the mosquito or to the marmoset. Presumably the critical factor in success or failure in maintaining the cycle was the amount of virus circulating in the infected marsupials. Perhaps the initial seven passages through rhesus monkeys in the first experiment had modified the virus away

from *M. nudicaudatus*, a condition that was gradually reversed by repeated transfer through marmosets. The significance of the role of the mosquito in these transfers must still be considered to be open to question. Whitman and Antunes (1938*b*) concluded that there had been no significant change in the M.A.J. strain as the result of the use of *A. aegypti* in passaging the virus from rhesus to rhesus.

While it is still uncertain whether or not serial mosquito passages may modify a strain of virus in a way favorable to the mosquito, there is evidence that the serial passage of virus strains through mouse brains or chick embryos results in a type of virus that is poorly adapted to development in mosquitoes. Working with yellow fever virus that had been modified by a varying number of serial passages through the brains of mice, Davis, Lloyd, and Frobisher (1932) found that although *A. aegypti* could transmit the virus in the 22d passage by bite, transmission was obtained less easily with later passages, and it was very difficult to maintain the virus in cyclic propagation through monkeys. For these latter experiments the French strain of yellow fever virus in the 119th to the 181st passage in mice was used. Roubaud and Stefanopoulo (1933) readily transmitted yellow fever virus by *A. aegypti* after 21 mouse brain passages, but failed to transmit the same type of virus after 147 and 172 passages in mice. Peltier, Durieux, et al. (1939*a*) again studied this problem in relation to the use of the living French neurotropic virus as a vaccine. On one occasion they infected specimens of *A. aegypti* by feeding them on a volunteer who had received a vaccine prepared from the 235th to 236th mouse brain passage. These mosquitoes failed to transmit virus by bite, although they were shown to contain the virus by injection of their bodies into a rhesus monkey. Other attempts to infect mosquitoes by feeding them on vaccinated persons failed. More recently Bates and Roca García (1946*c*) attempted to transmit neurotropic virus by means of mosquitoes of the *Haemagogus* genus but without success. They used French neurotropic virus in the 250th mouse brain passage. From the reported level of virus in the monkeys on which the mosquitoes fed, it is probable that the failure to transmit the virus was due as much to inadequate virus as to mosquito nonsusceptibility. However, in one experiment the blood virus level was over 10,000 infectious mouse doses per 0.03 cc., a concentration that should be adequate to promote infection in at least a few of the mosquitoes should they be susceptible to the strain of virus employed.

Another form of virus modification due to continuous passage in a dif-

ferent medium is the 17D strain of yellow fever virus which has been adapted to the chick embryo. This strain has been widely used by both The Rockefeller Foundation and the United States Public Health Service as a vaccine because of the fact that it has lost its capacity to damage cells or organs of vital necessity to man. As this vaccine is also a living virus which multiplies in man and may be demonstrated in the circulating blood, it was of practical importance to learn whether mosquitoes could become infected with it and transmit it. Roubaud, Stefanopoulo and Findlay (1937) attempted to infect specimens of *A. aegypti* by feeding them on monkeys that had been inoculated with similar culture viruses of several varieties grown in mouse embryo tissue or chick embryo tissue. They even passed the embryo culture virus through two passages in monkeys by intrahepatic inoculation. In no case did they infect the mosquitoes. Whitman (1939), working with the 17D culture virus, also failed to infect specimens of *A. aegypti* by feeding them at appropriate intervals on vaccinated humans or monkeys but concluded that the circulating virus levels were too low to give significance to these findings. What was more important, he showed that although the mosquitoes could be infected by immersing them while in the larval stage in concentrated suspensions of this virus, they nevertheless were unable to transmit the virus by bite.

Thus we have evidence that the prolonged maintenance of yellow fever virus in unusual tissues markedly reduces its capacity to establish itself in mosquitoes. The effect of shorter periods of passage in such tissues is more debatable. Davis, Lloyd and Frobisher (1932) and Roubaud and Stefanopoulo (1933) infected *A. aegypti* with 22d and 21st mouse brain passage virus respectively and obtained transmission by bite with apparently little difficulty, yet Bates and Roca García (1946*b*) gave evidence that even six mouse brain passages may reduce the capacity of a yellow fever virus strain to infect mosquitoes of the genus *Haemagogus*. In their study there is a suggestion that the lower infection rate in these mosquitoes could be explained by the fact that there was less virus in the circulating blood of the monkey receiving the mouse-adapted strain than in the blood of the monkey receiving the unmodified control virus. However, even if the six mouse brain passages modified only the behavior of the virus in the monkey, the secondary effect of a lowered infection in mosquitoes is still important.

POSSIBILITY OF VIRUS PASSAGE FROM MOSQUITO TO MOSQUITO

As indicated earlier, Aragão and da Costa Lima (1929a), Hindle (and Davis and Shannon (1930) found that the virus of yellow fever w tributed throughout the body of the infected mosquito. Thus it migh assumed that all tissues were infected. It was therefore thought possible the virus could be passed from mosquito to mosquito either through egg or at the time of copulation, or even by having larvae feed on the bod of infected females that had died in the water container in which the lar were growing. Of primary importance was the question of virus pass from generation to generation transovarially, particularly as Marchoux an Simond (1906a) claimed to have transmitted yellow fever to a human volu teer by the bite of a specimen of *A. aegypti* bred from the egg of an infecte female. However, Marchoux and Simond's data leave room for doubt to the validity of the observation, and studies by Philip (1929b), Davis and Shannon (1930), Frobisher, Davis and Shannon (1931), Kerr and Hayne (1932) and Whitman and Antunes (1938a) have failed to confirm them.

With regard to other methods of maintaining yellow fever virus in mos quitoes without recourse to passage through vertebrates, Aragão (1929b) re ported experiments with *A. aegypti* in which the virus was transferred from infected females to normal males and vice versa. He suggested that virus could be maintained in mosquitoes by this means for longer than the life span of a single female. Hindle (1930) confirmed the observations of Aragão that males could be infected by females but presented evidence that in this transfer of virus from female to male the males were only coated with the virus presumably present in the dejecta of the females, and were not truly infected. They could be rid of the virus by washing with physiologic salt solution. Frobisher, Davis and Shannon (1931) showed that virus sur vived in a colony of *A. aegypti* only during the life span of the originally infected females. Kerr and Hayne (1932) and Whitman mated infected fe males with normal males without evidence of transfer of virus, and Whit man also mated males infected as larvae with normal females without demonstrable virus transfer.

It is of interest at this point to note that Aragão kept his mosquitoes in glass tubes. Periodically these mosquitoes were given blood meals and were observed to excrete drops of clear fluid, presumably blood ser

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from the rectum This was the material that he called feces and that he showed to contain virus Obviously, this serum deposited on a glass surface would preserve virus from rapid death and presumably could contaminate the feet of noninfected mosquitoes put in the same tube Hindle apparently used pots in which he kept his *infected female mosquitoes and normal males* Unfortunately he didn t state whether he gave supplementary blood meals to aid in producing the excretion of serum with virus In contrast, the studies of Frobisher Davis and Shannon, Kerr and Hayne, and Whitman were done with mosquitoes in fairly large cages This perhaps would tend to reduce the contact of the noninfected males with the excreta, and the *excreta would dry faster However, on two occasions* Kerr and Hayne allowed the infected females to engorge on a monkey shortly before the normal males were liberated with them Although mating occurred at a *time when fluid droplets were being ejected* no males were infected

Marchoux and Simond (1906*a*) first suggested the possibility that infected females might die at the time of oviposition and fall into the water Subsequently, *larvae could ingest the liberated virus or virus* containing tissues and become infected Their own experiments, however, gave negative results

Davis and Shannon (1930) failed to infect larvae with yellow fever virus by putting the crushed bodies of infected females into the larval rearing pans, but Whitman and Antunes (1938*a*) found that if larvae of *A aegypti* were immersed in sufficiently potent virus suspensions, they would become infected and maintain their infection through the pupal stage into adult life Presumably this method of introducing the virus into the mosquitoes produced an infection in them similar to that acquired by adults in feeding Males were titrated at emergence and at subsequent periods and showed an increase in virus content Probably a short incubation period was needed before the females could transmit the virus by bite One lot of *A aegypti* failed to transmit the virus by bite when 1 to 4 days old but successfully transmitted 1 week later, another lot transmitted when first tested at 4 days of age Whitman also infected *Aedes scapularis* and *A leucocelaenus* in the larval stage In the case of *A scapularis* there was again a suggestion that a short incubation period was necessary in that when the females first fed at 2 to 3 days of age, no infection took place, whereas the same lot transmitted by bite a week later when the females were 9 to 10 days old Specimens of *A leucocelaenus* transmitted virus when first tested at 3 to 5 days of age It is of interest that an attempt to infect *Aedes taeniorhynchus* in the

larval stage failed In this experiment mixed *A taeniorhynchus* an *aegypti* larvae were placed in the same virus suspension Adult males females of *A aegypti* from this lot were infected but adults of *A tae rhynchus* contained no virus that was demonstrable either by bite or by oculation Unfortunately no further studies of this nature have been ported It would be of interest to learn whether nontransmitters are ins ceptible to infection in the larval stage as opposed to transmitters

VIRUS DOSAGE NECESSARY TO INFECT MOSQUITOES

The preceding sections have dealt with the response of mosquitoes to the ingestion of amounts of virus more than adequate to promote infection However such large amounts of virus are not necessarily available to mos quitoes in nature Yellow fever virus is present in the blood stream of an infected animal for only a few days and is at a maximum quantity for a still shorter period of time Furthermore some species of animals under going infection with yellow fever virus may never achieve the concentration of virus in the blood stream that is demonstrable in most of the primates Needless to say it is of importance to determine whether such animals can serve to infect mosquitoes or other arthropods in nature Studies on the response of mosquitoes to graded virus doses are therefore of considerable interest to the epidemiologist

Bauer and Hudson (1928*b*) noted that mosquitoes might fail to become infected when fed on a yellow fever patient even though the blood they ingested contained demonstrable virus when inoculated into a susceptible animal Later Bauer and Mahaffy (1930*a*) noted a similar situation when feeding *A aegypti* on infected monkey blood Hindle (1930) attributed this to the concomitant presence of antibodies and virus which presumably resulted in the neutralization of the virus in the mosquitoes stomachs With the development of the use of mice for titrating the amount of virus in the blood stream more accurate determination of the threshold of infection became possible Whitman (1937) was able to produce infection in speci mens of *A aegypti* fed on monkeys whose serum contained as little Asibi virus as 1 800 and 570 minimal infectious doses (M I D) for mice Inasmuch as these infectious doses are in terms of 0 03 cc of serum it may be assumed that the mosquitoes actually engorged from 1/10 to 1/40 of this amount of virus In Whitman's experiments no attempt was made to dete

proportion of mosquitoes infected to the total number feeding but it is probable that only a limited percentage was actually infected

Bates and Roca García (1946b) made an intensive study of this question in Colombia using *H spegazzinii falco* infected with either the Rodas or the Perez strain of virus Following an appropriate incubation period individual mosquitoes were tested for virus content by inoculation into baby mice Unfortunately the strains of virus used in these studies are not so highly pathogenic for mice as the Asibi virus and thus the actual concentration taken up by the mosquitoes in terms of infectious particles is not known It is known however that more virus was ingested than the following figures indicate The authors found that when the quantity of virus in the circulation of the donor monkey was so small as to infect no mice at a dilution of greater than 1 10 no mosquitoes were infected With enough virus to infect one or two mice in a dilution of 1 100 one of six and one of 10 mosquitoes tested individually by injection were found to be infected With still more virus detectable in mice in a dilution of 1 1 000 one of 11 and three of seven mosquitoes were infected With greater quantities of virus detectable in a 1 10 000 to 1 100 000 dilution virus was recovered from a majority of the mosquitoes tested and with still greater concentrations from over 90 per cent of them Anderson and Osorno Mesa (1946) in a report of their studies on the capacity of *Haemagogus splendens* to transmit yellow fever gave data not unlike those of Bates and Roca García In their work another Colombian virus was used that was fully pathogenic for mice permitting accurate quantitative determinations With dosages in the neighborhood of 300 M I D for mice none of the mosquitoes became infected When the blood contained about 1 000 M I D very few mosquitoes were infected but when it contained 20 000 to 30 000 M I D 5 per cent and 22 per cent of the mosquitoes tested were infected In these experiments of Bates and Roca García and Anderson and Osorno Mesa the mosquitoes were tested by injection into mice

In contrast with the preceding results Waddell and Taylor (1947) apparently had difficulty in infecting mosquitoes when the virus content of the blood stream was less than 1 000 000 M I D It is possible as the authors point out that some of the failures to infect mosquitoes at a time when less virus was in circulation may have been due to the fact that the test for infection was the capacity to transmit by bite over a relatively short time rather than by injection As previously pointed out Bates and Roca García found that many infected mosquitoes have prolonged incubation

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It has been noted particularly when the titer of the circulating virus in t animal host is low that mosquitoes may be more frequently infected whe fed upon an animal during the early stage of the disease than later when th circulating virus is on the wane This has been attributed to the develop ment of antibodies that curtail transmission of the infection to the vector

In attempting to summarize these observations it may be said that whe the virus content of the blood stream of an infected animal is of the order of 100 or less M I D for mice per 0 03 cc (the volume of serum inoculated into mice) it is unusual for a single mosquito to become infected With blood virus levels of about 1 000 M I D a limited number of mosquitoes feeding will become infected This in turn will be influenced by the tem perature At low temperatures a greater proportion of the mosquitoes will eliminate the virus without becoming infected As the virus content of the blood increases beyond 10 000 M I D the percentage of infected mosquitoes will rise rapidly and will probably not be influenced by temperature as far as the infection of the mosquito is concerned although the extrinsic incu bation period will obviously be influenced

EFFECT OF IMMUNE SERUM ON VIRUS IN THE MOSQUITO

Aside from the effect of temperature and virus dosage on the develop ment of yellow fever virus in mosquitoes it was of interest to the early workers in this field to learn whether the infection in the mosquito could be modified by the ingestion of blood from primates immune to yellow fever Obviously if infected mosquitoes could be rendered virus free by feeding on an immune animal this would have considerable bearing on epidemiology Dinger Schuffner et al (1929) first explored this possibility and concluded that once infected a mosquito is not sterilized by ingesting serum of a person immune to yellow fever Furthermore they noted that the ingestion of immune serum did not prevent a mosquito from becoming infected with virus at a subsequent time Hindle (1930) confirmed these observations However he found that under certain circum

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FI F E C T O I I M M U N F S E R U M O N V I R U S I N T H E M O S Q U I T O

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tures of virus and immune serum in the stomach of a mosquito would prevent the virus from becoming established. He believed this to be the explanation of failures to infect mosquitoes fed on a monkey in a late stage of yellow fever even though the blood of the animal still contained detectable virus as shown by inoculation into a susceptible monkey. N. C. Davis (1931a) in turn confirmed the findings of Dinger, Schüffner, et al. and of Hindle, although he was unaware of the latter's work at the time of his own experiments. In view of the evidence that immune serum can neutralize virus in the stomach of a mosquito, it would be of interest to know whether this effect could still be demonstrated during the first 2 or 3 days after the ingestion of virus, prior to its multiplication in the mosquito tissues. Unfortunately, this point has not been investigated.

AMOUNT OF VIRUS INJECTED BY A MOSQUITO

A question of interest that should be raised before this brief survey of the relationship between virus and mosquito is brought to a close is: How much virus is introduced into an animal by the bite of one infected mosquito? Davis (1934c) is the only person who has reported experiments on this problem. Unfortunately, these experiments, of necessity cumbersome, are not conclusive. However, they indicate that mosquitoes inject at least 100 infectious doses for monkeys, if not more. Whether the amount would be affected by temperature is not known.

RELATION OF MOSQUITO ECOLOGY TO TRANSMISSION OF YELLOW FEVER

BREEDING FOCI

It is obvious that a mosquito, to be of epidemiologic importance, must come in close contact with susceptible vertebrates. Dinger, Schüffner, et al. (1929) demonstrated that *Aedes albopictus* from Malaya could transmit yellow fever virus. Roubaud, Colas-Belcour, and Stefanopoulo (1937) transmitted the virus with *Aedes geniculatus* from France, and Bennett, Baker, and Sellards (1939) were successful with *Aedes triseriatus*, a tree-hole breeding species from the northeastern section of the United States. Inasmuch as these mosquitoes do not come in contact with yellow fever virus, they have no actual epidemiologic significance. An equally effective separation of

mosquito and virus may occur within a smaller geographic area For example coastal mosquitoes breeding in brackish water have little or no contact with the vertebrate population even a relatively short distance away in the interior The ecology of mosquitoes therefore plays an important role in determining whether or not an experimentally proved transmitter is of epidemiologic significance

The geographic location of mosquito species is the result of selection on the part of individual species and reflects their choice of suitable waters in or near which to lay their eggs In general there are two major groups of mosquitoes those whose eggs must hatch immediately and those whose eggs can withstand long periods of drying In the first group conditions must be such that either the aquatic forms or the adults can survive adverse climatic conditions to produce the next generation with a return of favorable conditions In contrast the adults of those species whose eggs are resistant to desiccation do not of necessity have to survive adverse conditions in the adult or aquatic stages

Aside from this generalized division there are a variety of classified breeding loci which have a bearing on the prevalence of a particular species in a geographic location at a specific season These may be roughly divided as follows (*a*) temporary or permanent surface water (*b*) natural containers (Shannon 1931*a*) such as tree holes bamboo leaf axils of certain plants epiphytic plants fallen leaves and nutshells (*c*) artificial containers

In general mosquitoes that avail themselves of temporary surface water are of the group laying eggs that are resistant to drought With the onset of rains the eggs already present in the ground hatch in large numbers and rapidly produce a prodigious number of adults With the cessation of rains and the drying up of the preferred breeding waters these mosquitoes quickly diminish in abundance almost to the vanishing point While such mosquitoes might play a part in the transmission of yellow fever it is to be expected that they would be associated with epidemic outbreaks rather than with the endemic month to month transfer of the virus As an example of this group we have *A scapularis* in Brazil This mosquito is an excellent experimental vector and on occasion has been found in abundance associated with epidemic jungle yellow fever However no true evidence of its articipation in the dissemination of yellow fever has been produced

As a variant of the temporary surface water there are such breeding places s rock pools in stream beds These can be washed out by floods or dried up y drought and they are therefore potentially temporary even in

seasons Among the mosquitoes breeding in this type of surface water is the potential vector *Aedes fluviatilis* in Brazil which transmits yellow fever readily in the laboratory but has never been shown to be associated with outbreaks of jungle yellow fever Other types of rock pools not associated with stream beds are also used by mosquitoes While less subject to being washed out they are otherwise similar Such rock pools are the favored habitat of *Aedes vittatus* a potential vector of yellow fever in Africa

Among the mosquitoes breeding in permanent surface water which include predominantly the genera *Anopheles* and *Culex* and which would be expected to maintain a relatively continuous density it so happens that no species truly susceptible to yellow fever has been found

The genus *Taeniorhynchus*[2] is in an intermediate position in that it breeds in permanent surface water but the larvae attach themselves to the roots of aquatic plants for purposes of respiration Thus their season of maximal density corresponds to the presence of the plants and frequently occurs at the end of the rainy season In the absence of the plants there is no breeding

It is among the fauna breeding in tree holes that the bulk of the experimental yellow fever vectors are to be found The eggs of the majority of the mosquitoes of this group are resistant to drying and are deposited on the damp wood just above the water level During the next few days the embryo develops up to the point of hatching then passes into diapause As the water level falls the eggs dry without harm to the contained embryo Following rains the eggs are flooded and some of them rapidly hatch and produce adults Those that fail to hatch however are still viable and after a second drying and second wetting another brood of larvae is produced This can go on for several cycles without more eggs being laid in the same tree hole In this manner tree holes can produce a small but spread out population of mosquitoes Furthermore they are not so likely to be washed out as are rock holes nor are they subject to rapid evaporation before the emergence of adults as are temporary surface pools Needless to say tree hole breeders are intimately associated with forest inhabiting vertebrates In this group are the genus *Haemagogus* and such important *Aedes* as *A leucocelaenus* in South America and *A africanus* in Africa—definitely established vectors of yellow fever in the forest

Bamboo breeders may be divided into two groups those that breed in the broken stubs and those that breed in the internodes of the growing plant

[2] See footnote p 231

In South America there is little overlapping between the two fauna However some of the tree hole breeders may occasionally be found breeding in broken bamboo stubs In particular this is true of *A leucocelaenus* and to a lesser extent of *Haemagogus* species Open stubs of bamboo are similar to tree holes except that they are frequently of small volume and hence subject to more rapid evaporation and are more often dry

The mosquito fauna inhabiting internodes in intact bamboo are rather restricted numerically Entrance of the female mosquito into the segment for oviposition is usually by way of beetle holes In South America this type of breeding is characteristic of some of the sabethines which up till now have given no evidence of playing a part in the spread of yellow fever

The plant breeding fauna is extensive and under certain circumstances very abundant This too may be divided into two types The first type prefers to breed in the collections of water to be found at the bases of leaves of such plants as the banana and colocasia which are frequently peridomestic having been artificially cultivated In general such breeding places are relatively near the ground The second type breeds in the water contained in epiphytic plants either in the broad leaf bases or in a central water container Insofar as these plants may be terrestrial or may be found at very great heights when attached to the branches of forest trees they are inhabited by a wide variety of forest mosquitoes In general they are not associated with human habitation although in Trinidad *Anopheles (Kerteszia) bellator* which breeds in the epiphytes in the shade trees required for the cocoa plantations has caused a malaria problem In this group of plant breeders one species *A simpsoni* is of interest In certain areas of Africa it is found breeding predominantly in the peridomestic bananas and colocasia in immediate contact with the local human population Not only is it an efficient laboratory vector of yellow fever but it has been found infected in nature

Finally there are the mosquitoes that breed in artificial containers such as discarded cans bottles watering troughs and funeral urns Of these *A aegypti* is undoubtedly the most important As receptacles of this kind are closely associated with man any mosquito breeding in them is bound to be peridomestic and peridomesticity means intimate contact with man If such a mosquito can transmit yellow fever it is almost certain to be of importance in the epidemiology of the disease Obviously therefore because of its breeding habits and its susceptibility to the virus of yellow fever *A aegypti* is an outstanding vector of the disease as it occurs in urban areas

On the other hand such a restricted breeding locus would prevent its playing a part in the dissemination of the disease in the forest Certain mosquitoes normally considered nondomestic may occasionally acquire domestic habits Soper and Serafim (1933) noted *A fluviatilis* breeding in ant rings and Komp and Kumm (1938) reported finding the larvae of *Haemagogus anastasionis* in funeral urns in Costa Rica

FEEDING HABITS

The breeding habits of a mosquito will determine whether it is abundant or scarce at any selected period of time and whether it will be in close proximity to or segregated from the vertebrate inhabitants of an area However the mere presence of a mosquito in an area does not indicate that it is actively engaged in biting the vertebrate inhabitants Several factors are *in operation to determine the degree of contact between the mosquito* and any particular vertebrate

In the first place there is the matter of blood preferences As is well known female mosquitoes take a blood meal for the purpose of maturing their ova their other requirements are cared for by the ingestion of plant juices Members of the genus *Megarrhinus* require no blood meals and in fact their mouth parts are so formed as to render them incapable of bloodsucking Certain other genera while mechanically capable of biting have not been observed to do so Nevertheless most mosquitoes take blood and therefore are potentially of interest to the epidemiologist Among those taking blood many have preferences that influence their contacts with any one species of vertebrate *For example* certain *anophelines prefer* feeding on animals rather than on man and may be diverted from man by an animal barrier Other mosquitoes particularly *Culex* species are more prone to feed on birds These food preferences are probably of greater importance in the final clarification of the epidemiology of yellow fever than is as yet realized for while it is reasonably easy to determine what species of mosquitoes in an area will bite man it is not known which mosquitoes will bite other susceptible vertebrates

Aside from food preferences there are what might be called altitude preferences This was first observed in Colombia (Bugher Boshell Manrique et al 1944) In 1940 Boshell Manrique was watching woodmen fell a tree *Subsequent to its fall he found himself being bitten by mosquitoes* of the genus *Haemagogus,* which were rarely seen at that season in that area This

suggested that these mosquitoes did not die out by that time of year as had been supposed but rather retreated to the forest canopy Experimental captures in the trees soon confirmed his suspicion The question of vertical distribution has an important bearing on the contact of a species of mosquito with arboreal as opposed to terrestrial vertebrates Furthermore unless mosquito captures have been made at different altitudes it is quite possible that observed densities of any one species may not reflect the actual density at that time

Another factor related to the feeding habits of a mosquito must be taken into account namely the hours of the day or night at which its peak feeding occurs For example in epidemics of jungle yellow fever in Brazil it was found that most of the human infections were in males who entered the forest by day frequently at the noon hour to seek shade while eating lunch This was found to coincide with the peak feeding activities of mosquitoes of the genus *Haemagogus* On the other hand such a period of maximum feeding activity would theoretically result in a minimum contact with any nocturnal vertebrates which became inaccessible to mosquitoes by day

In contrast with the midday activity of mosquitoes of the *Haemagogus* genus is the sharply restricted crepuscular activity of *A africanus* This mosquito confines its major feeding period to about one hour of the day after sunset but before complete darkness This is an ideal period for feeding on monkeys that are settling down for the night although conceivably too early for feeding on strictly nocturnal vertebrates It is worth noting in this connection that in forested areas even the terrestrial monkeys sleep in trees and are equally subject to being bitten by arboreal mosquitoes

The question of flight and biting activity is one that has long interested medical entomologists to a considerable degree although they are still not certain as to the physiologic mechanisms involved While it is obvious that each species of mosquito has evolved an activity pattern that superficially may be related to the degree of illumination recent work by Bugher would indicate that periodicity is so well established in the mosquito that artificial changes in illumination fail to alter the pattern Activity of the mosquito will occur at the appointed time regardless of the conditions of light in the artificial environment in which it is placed Under the circumstances light alone may not be the dominant factor This is of potential interest in the hypothetical case of a day flying tree hole breeding mosquito entering a tree hole for the purpose of oviposition and encountering a suitable blood meal Would it respond by feeding despite the suddenly reduced light intensity?

Would the urge to deposit eggs influence the feeding response? The answers to such questions are not known yet they might reveal important modifications in the period of contact between mosquitoes and vertebrates particularly when the mosquitoes are diurnal and the vertebrates nocturnal

FLIGHT RANGE

Another ecologic factor deserves mention at this point *i e*, the flight range of mosquitoes It is evident that the geographic spread of yellow fever must be explained on the basis of the dispersal of either the susceptible vertebrate or the insect vector In the case of man particularly in modern times this method of spreading yellow fever is of great potentiality On the other hand results of studies on monkeys and other forest vertebrates indicate that they are greatly restricted in their range As for insect dispersal the story of the spread of yellow fever to the temperate zones from the tropics by means of ships carrying infected specimens of *A aegypti* is only too well known However there is considerably less information on the spread of jungle yellow fever in the forest Shannon Burke and Davis (1930) conducted a series of experiments in Brazil using marked specimens of *A aegypti* liberated in houses Dispersion began within 24 hours but was not wide and in one experiment 66 37 per cent of the mosquitoes were recaptured in the house of release and only 3 12 per cent in adjoining houses The greatest reported distance traversed was 120 m Shannon and Davis (1930) in more extensive studies in villages noted extreme ranges of 300 m However in one experiment 12 000 stained specimens were released on a boat 300 m from one shore and 900 m from the other Eight mosquitoes were retaken 950 to 1 000 m from the boat (It is of interest that all the mosquitoes were captured to the west—the 900 m distance) Thus *A aegypti* in South America does not travel far More recently Bugher and Taylor (1949) have attacked the problem of the dispersal of *A aegypti* using radioactive phosphorus and strontium as the means of marking their mosquitoes This ingenious method of marking did not apparently shorten the life span of the mosquito yet facilitated detection by means of a Geiger counter No manipulation of the mosquito before release was required Using this method a maximum travel of 3 800 ft was recorded The authors note that the mosquitoes were distributed largely by wind drift rather than their own flight although the latter contributed

With regard to forest mosquitoes in South America Causey and Kumm

(1948) and Causey Kumm and Laemmert (1950) have recently reported studies on marked mosquitoes with particular emphasis on those of the genus *Haemagogus* and on *A leucocelaenus* The marking was done with bronzing powders In these studies a specimen of *Haemagogus spegazzinii* was captured as far as 11 5 km from the point of release and specimens of *A leucocelaenus* up to 5 7 km from this point Other extreme flight ranges were *A serratus* 11 5 km *P ferox* 10 8 km *Wyeomyia* sp 5 7 km *Aedes terrens* 5 6 km and *Chagasia* sp 2 3 km The longest survival recorded was that of a specimen of *P ferox* captured 55 days after release Again the direction of flight was apparently influenced by prevailing wind

LONGEVITY

A final ecologic factor of importance is the longevity of mosquitoes It is almost impossible to determine the average survival time of mosquitoes in nature In fact if we were to take some of the figures reported no malaria or yellow fever could exist in view of the brief life span of the mosquito hosts As these diseases are with us in reasonable abundance the average duration of life of the mosquitoes must be adequate to cover the requisite periods of incubation In studies on the survival rates of caged specimens of *A aegypti* Beeuwkes Kerr et al (1933) showed that these were influenced considerably by such environmental factors as temperature and humidity They compared the survival of these mosquitoes in northern Nigeria (Gadau) and in southern Nigeria (Yaba) In Yaba which has relatively uniform temperature and humidity curves it was found that the mean life expectancy of the females varied from 70 to 116 days with a maximum of 131 to 225 days At Gadau however this varied With higher temperatures and lower relative humidities the mean varied from 10 to 22 days with a maximum of 26 to 45 days As the temperature fell and the relative humidity rose means of 40 to 56 days were found with a maximum of 71 to 109 days With still lower temperatures and again lower humidity the survival time fell off again This could be significantly increased by putting the mosquitoes in a moist chamber Thus longevity is not purely a species characteristic but is significantly modified by environment Putnam and Shannon (1934) making similar studies in Brazil found that a lot of 1 000 caged *A aegypti* females given frequent blood meals had a mean life expectancy of 62 days In 1 000 females that were not fed blood this rose to 82 days In both groups the maximum life span was 112 days The authors said it is

impossible to apply these figures to natural conditions They noted that Connor (1924) suggested that all infectious females will disappear in 6 weeks *following rigid antilarval campaigns* However they felt that the interruption of transmission indicates only that the mosquito population has been reduced to a point where the probability of transmission has become nil The number of females still surviving may be considerable In view of these observations the recapture of a stained specimen of *P ferox* 55 days after its release is of considerable interest particularly as the group of dyed mosquitoes in which this specimen was included was made up of wild caught adults of obviously undetermined age accumulated over a period of 3 weeks before being sprayed and released

SPECIES OF MOSQUITOES OF INTEREST IN RELATION TO YELLOW FEVER

In the preceding sections of this chapter it has been pointed out that a species of mosquito is important from the standpoint of yellow fever dissemination if it is susceptible to infection with the virus of the disease if it is known to transmit the virus and if its habits of breeding and feeding *are such as to associate it with susceptible vertebrates* It will now be worth while to summarize what is known of the particular species that have been shown either in laboratory studies or in field investigations to be the ones *most intimately connected with the dissemination of the virus* *Inasmuch* as the important species of South America differ from those of Africa data for the two areas will be discussed separately

IMPORTANT SPECIES OF SOUTH AMERICAN MOSQUITOES

Table 7 lists the species of South American mosquitoes that have been studied in the laboratory as possible vectors of yellow fever The selection of mosquitoes for study has been dictated both by epidemiologic findings and by convenience *It is quite obvious that the list of species is at best fragmentary* particularly with regard to such abundant groups of mosquitoes as the tribe *Sabethini* and the genus *Culex* This is due to difficulties in classification as well as to *the difficulty of maintaining these mosquitoes in* the laboratory and getting them to feed on experimental animals Furthermore limited experimentation (with a few exceptions) has indicated that

TABLF 7

SPEC OF SOUTH A ER CAN MO Q TOE S D FD N HE LABOR TOR

Mo q P	R fer n	R
A d a gyp	Un d S a e 6 Congre Ye ow Fe e 1911	
A d nub u	Wh man and An une 1937	T
A d ap ri	Da and Shannon 1929	S
A d erratu	D vi and Sh nnon 19 9	T
A d err n	D and Shannon 193 b	S
A d fuh hora	Davi a quo ed b Wh m n and An une 1937	S
A d flu at	Davi and Shannon 1931a	S
A d a n hyn hu	D vi and Shannon 1931a	T
A d leu a nu	Wh man and Antune 93	
Ha mag gu ap n	Shannon Wh man F an a 938 Wadd 949	S
Ha mag gu qu nu	Shannon Wh man F an 938	
Ha mag gu pega n	Wadd and Kumm 1948	T
Ha mag g p ga n fa	Wadd I and Ta o 1945 An une and Wh man 937	T
	I mm d Ca ro Fe re a Ta o 946	T
Ha mag gu plend n	Bughe B h Manrique e a 1942	I
Ha mag gu u a	Ba and Roca Garc 1945 194	
P phora fer x	And rson and Oso no 1946	
P or ph a gula a	An un and Wh m n 1937	T
Ta n hyn h a b a	Da and Shannon 93 b	T
Ta n hyn hu h y on um	Wh man and An une 1937	S
Ta n hyn hu fa a a	D and Shannon 1931b	S
Ta n hyn hu ux aman n a	Da and Shannon 931b	S
Ta n hyn h n	D and Shannon 1931b	S
T h pr op n d g a m	Wh man and An une 1937	S
	Kumm and F ob h 1932	S
T h pr p n f n u	D and Shannon 1931b	S
I y my a br m arum	Kumm and Frob h 1932	S
I y my a ob a	Wh man Tu ock and Wadd a quo ed b W dd 949	N †
L ma u du ham	Da and Sh nnon 1931a	T
C x fa gan	Da and Sh nnon 1931a	N
C n gripa p	D and Shannon 193 a	N
An ph alb ar	Da and Shannon 19 9 1931b	N
An ph ar ma u	D 1933b	T ‡
	Dav a quoted by Wh man and An un 1937	
	Da and Shannon 1931b	S §
	D and Sh nnon 1931b	N

T = an m by b e S = u ep b n h re a n viru n h body o rea onab ong period of me N = no u p b e ru ap d m na d from he body

† D v and Shannon repo d on one pecimen Kumm and Frob he po d on wo pec m n Found nf ed n na ure

‡ D P re he e e repo by D v and Shannon (1931b) ha th pec was qu s onab y us. Th no uffi n e d n e o be con d red fina

§ Al hough a w pecim n were ab o re n ru n h bod fo m re han a man d m lo (933b was abe o g an m on by b e w h h pecie In the experimen um b e A a who h e of C fa gan ap d y o h u ome re a n d and o he an m ted b hem nd hemse e o h ru comp y mu be con d red n effi n ecto

most of these species are not susceptible. This has tended to discourage a more extended investigation.

As may be seen from Table 7 the number of mosquitoes that have been shown capable of transmitting yellow fever is small and almost entirely restricted to species of the genera *Aedes* and *Haemagogus*. The only other species of mosquito that has been tentatively considered a possible vector is *Trichoprosopon frontosus*, one of the vast tribe of *Sabethini*—perhaps the predominant tribe of forest mosquito throughout large parts of South America. The proved and potential vectors will now be discussed one by one.

Genus *Aedes*

Aedes (Stegomyia) aegypti Linnaeus

Ever since the original studies of the Reed commission (United States 61st Congress, Yellow Fever, 1911) all workers investigating yellow fever have used *A. aegypti* as the model of vector efficiency. Stokes, Bauer and Hudson (1928*b*) demonstrated its capacity to transmit the infection from monkey to monkey in the laboratory as soon as they had proved the susceptibility of rhesus monkeys to experimental infection. The extrinsic incubation period, crudely indicated by the Reed commission, was more accurately determined by Bauer and Hudson (1928*a*), and the effect of temperature thereon was studied by Hindle (1930) and by N. C. Davis (1932*a*). All this work confirmed opinions already established through epidemiologic investigation of classic urban epidemics of yellow fever.

More important than these studies were the ecologic findings. *A. aegypti* in the Americas is restricted to a peridomestic environment, breeding in close proximity to, if not actually inside, human habitations. Shannon (1931*a*) liberated specimens of this mosquito in the forest, but they failed to maintain themselves. This intense association with man has been of primary importance in two ways. In the first place, it made an ideal situation for the transmission of urban yellow fever in epidemic form. Every yellow fever patient had the maximum possible contact with the vector, which in turn had the maximum contact with susceptibles. Furthermore, as the mosquito bred readily in the bilge water of ships, as well as in the drinking water containers, any case of yellow fever occurring aboard ship was a source of infection to the ship's mosquito population, which could be transported to many distant ports. On the other hand, the close contact

with human habitations has rendered *A. aegypti* susceptible to c measures to a much greater extent than would have been the case if been less intimately associated with man. By screening such breeding tions as wells and cisterns, by eliminating accidental water containers as defective roof gutters, bottles and cans, and by periodically oiling water containers as could not be eliminated or screened, the density *A. aegypti* could be reduced to a point where transmission of yellow fe no longer occurred and in many places the species could be eradic entirely. The mosquito's habit of daytime resting in houses, coupled wi its tendency to stay close to its breeding focus further facilitated its contro Although Shannon and Davis (1930) recorded flight ranges of 1,000 m fo this species, most adults remain within 100 m of their point of origin. If adults are captured in a house, it can be assumed that a hidden breeding focus exists in the close vicinity. Usually this can be found without undue search.

It is of interest that by larval control alone city epidemics of yellow fever could be rapidly broken. Connor (1924) stated that within 6 weeks of the time that breeding was checked no more cases of yellow fever occurred. His findings imply that in nature a period of 6 weeks represents the significant life span of *A. aegypti*. However, as previously stated, this may be an underestimation.

In limited attempts to isolate virus from naturally infected specimens of *A. aegypti* in South America, N. C. Davis (1931*a*) was entirely unsuccessful. However, he pointed out that there is a good possibility that in suspensions of pooled mosquitoes the presence of recently engorged human immune serum in some specimens may have neutralized what virus there may have been in others.

Aedes (Ochlerotatus) scapularis Rondani

Interest in *A. scapularis* stems from Davis and Shannon's demonstration (1929*c*) that this species of mosquito could transmit yellow fever by bite in the laboratory. The interest was intensified in 1932 when an epidemic of yellow fever occurred in the Valle do Chanaan, Espirito Santo, Brazil, in the virtual absence of *A. aegypti*. The investigation of this outbreak reported by Soper, Penna et al. (1933) disclosed that mosquitoes of the species *A. scapularis* were particularly abundant near a house in which seven persons were infected. Although no actual evidence against this species was obtained, its probable role as vector was suggested. Later

Antunes (1937) verified Davis and Shannon's earlier report on the efficiency of the species as a laboratory vector

A scapularis breeds in temporary rain pools and, under suitable conditions may be found in great abundance As Shannon (1931*a* and *b*) pointed out it may be considered to be a facultative suburban mosquito rather than a strictly sylvan one As such, it is frequently captured in houses During the dry season it almost disappears In its choice of a blood meal it is relatively unselective and can be taken with ease with various vertebrate baits (Kumm and Novis, 1938, D F Davis, 1945) It is more readily captured at ground level than in trees Its feeding cycle is rather broad, and although its peak activity is in the late afternoon, specimens may be captured at almost any hour of the day or night

Despite the early interest in *A scapularis*, a naturally infected specimen has never been captured In the period of time during which Shannon, Whitman, and Franca (1938) captured infected specimens of *Haemagogus* and *A leucocelaenus*, a few specimens of *A scapularis* (180) were caught and injected into experimental animals, but without virus isolation Laemmert, de Castro Ferreira and Taylor (1946) also attempted to isolate virus from this species during their investigations of jungle yellow fever in Ilhéus but failed, despite the isolation of virus from *H spegazzinii* during this period

A scapularis has a wide geographic distribution, being reported from Mexico and the West Indies to Paraguay and Argentina on the south, and from Ecuador on the west to Pernambuco on the east However, the density of the species may be quite variable in any of these localities

Aedes (Taeniorhynchus) fluviatilis Lutz

As in the case of the preceding species, *A fluviatilis* was one of the first native South American mosquitoes that Davis and Shannon (1931*a*) showed to be capable of transmitting yellow fever by bite in the laboratory It was found to be present in the Valle do Chanaan during the epidemic of yellow fever in 1932, and Whitman and Antunes (1937) confirmed Davis and Shannon s findings on this species as an efficient laboratory vector

A fluviatilis breeds in rock pools and stream beds and may be classified as a facultative suburban breeder It was found by Soper and Serafim (1933) breeding in water filled cement ant rings, used to protect growing flowers in lawns However, throughout large parts of South America where yellow fever has occurred either endemically or epidemically, its favored breeding sites are either absent or inadequate to produce a significant den

sity of adults Because of its scarcity in the areas that have been intensiv studied there is little information concerning its altitude preferences hours of feeding that is based on modern methods of investigation Ho ever such data as are available indicate that the habits of this mosquito ar similar to those of *A scapularis*

A fluviatilis has been recorded from Mexico south to Panama and Colom bia as well as from coastal points in Guiana and Brazil as far south as São Paulo (Kumm 1931*a*) A few specimens were taken in the Passos area of Minas Gerais Brazil by Causey and dos Santos (1949)

Aedes (Taeniorhynchus) taeniorhynchus Wiedemann

Davis and Shannon (1931*a*) reported a single experimental transmission of yellow fever virus by the bite of *A taeniorhynchus* although in earlier studies the same authors (1929*c*) were unsuccessful with this species Whit man and Antunes (1937) failed to get transmission by bite with the species and were inclined to believe that the report of the positive result was ques tionable Later Whitman found in an isolated experiment that *A taenio rhynchus* larvae failed to take up virus in contrast to *A aegypti* larvae placed in the same virus suspension However the above mentioned authors are in agreement that adults of the former species can become infected by feeding on blood containing the virus and can retain the virus for long periods of time They must therefore be considered on the borderline of potential vectors

A taeniorhynchus has a wide distribution along the Atlantic coast ex tending from the New England states in North America to Argentina in South America However it is a coastal species and does not penetrate the interior In areas where members of the species are abundant they fre quently enter houses in search of blood meals

Aedes (Conopostegus) leucocelaenus Dyar and Shannon

It is of interest that *A leucocelaenus* has in the past been tossed back and forth between the genus *Aedes* subgenus *Finlaya* and the genus *Haemagogus* as it is clearly intermediate between the two Sha [illegible] (1931*b*) however while admitting the difficulty of accurate classification preferred its present taxonomic position

This species first achieved recognition as a vector of yellow fever when hannon Whitman and Franca (1938) captured wild [illegible] that hen brought to the laboratory transmitted yellow fever to [illegible]

by bite showing that they were not only acquiring their infection i but were capable of transmitting the disease Later Bugher Boshe rique et al (1944) and Boshell Manrique and Osorno Mesa (1944) c specimens of A leucocelaenus in Colombia that had become infe nature Subsequently Whitman showed that A leucocelaenus could fected in the larval stage and the resultant adults would transmit by More recently Waddell (1949) has assembled the available data from l on laboratory transmission experiments with this species and has show to be an effective vector

The species is very similar in its habits to the members of the ge *Haemagogus* to be discussed below It is a tree hole breeder lives in t forest and has a diurnal feeding cycle It is not however so intensely phot philic as members of the genus *Haemagogus* having a less sharp prefe ence for the noon hours Although it may be captured in the fores canopy its preference for this zone is much less marked than that shown by species of *Haemagogus* a greater proportion of specimens being caught at ground level In addition it is more adaptable in its choice of breeding locations being frequently found away from the true forest in scrub bam boo where it breeds readily in the broken stubs The author even found *A leucocelaenus* larvae in a metal capped box on the ground used to cover a water hose connection in which the faucet leaked providing an artificial ground pool A female had apparently entered through the finger hole in the metal plate in order to deposit her eggs

The distribution of this species has been recently summarized by Kumm and Cerqueira (1951*b*) It is widely spread throughout South America It is very prevalent in Brazil especially in the south where it is found to be much more common than *Haemagogus* species Despite its relatively low density in eastern Colombia naturally infected specimens have been cap tured there and it therefore presumably plays a part in the dissemination of yellow fever

It is of interest that this species is fairly common in the area of Panama n which human yellow fever was diagnosed in 1949 (Courtney 1950 and alindo Trapido and Carpenter 1950)

enus *Psorophora*

Except in the case of one experimental lot of *P ferox* studied by Davis reported by Whitman and Antunes (1937) no one has l

transmit yellow fever by the bite of members of this genus. In this instr[ance] Davis transmitted virus by the bites of the mosquitoes on two occasions. [As] has been stated earlier this observation is of interest in that it may indic[ate] a variability within a species as far as ability to transmit is concerned.

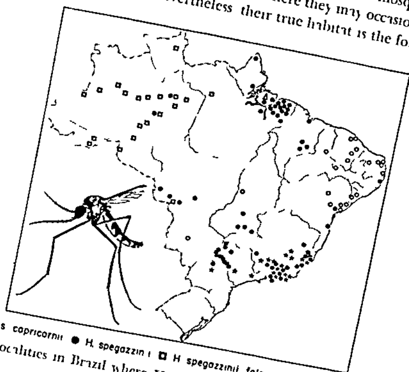
The genus is found as far north as Canada and south to Argentina. Th[e] subgenus *Janthinosoma* to which *P. ferox* belongs tends to be widesprea[d] in the forested areas of Brazil where yellow fever has occurred. Other species of this subgenus that may be locally abundant are *Psorophora lutzi* and *Psorophora albipes*. Such members of the genus *Psorophora* breed in temporary woodland pools and have a seasonal distribution much like that of *A. scapularis*, at times being abundant, at times virtually absent. They are daylight flyers.

Genus *Haemagogus*

Following the first isolation of yellow fever virus transmitted by the bite of wild-caught *Haemagogus* mosquitoes by Shannon, Whitman and Franca (1938) this genus became of particular interest to investigators of yellow fever in South America. However an early lack of knowledge of species characters led to considerable confusion in nomenclature. In this genus female characteristics are not adequate to separate species and it is necessary to examine males for final identification. Following virus transmission studies by Antunes and Whitman (1937), Shannon, Whitman and Franca (1938), Bugher, Boshell Manrique et al. (1944), Boshell Manrique and Osorno Mesa (1944) and Bates and Roca Garcia (1945 and 1946b), several taxonomic studies on the species identification of *Haemagogus* have been reported. Cerqueira and Lane (1945) and Kumm, Osorno Mesa and Boshell Manrique (1946) considerably clarified the situation by collecting females, allowing them to lay eggs in the laboratory and rearing males and females from these eggs. In this way they were able to reclassify several of the species of *Haemagogus*. They showed that the *Haemagogus janthinomys* of Antunes and Whitman was in reality *H. spegazzinii* and that the *H. capricornii* of Bugher, Boshell Manrique et al., Boshell Manrique and Osorno Mesa and Bates and Roca Garcia was in reality a subspecies of *H. spegazzinii*, namely *H. spegazzinii falco*.

All the *Haemagogus* species that have been implicated in yellow fever transmission are primarily tree hole breeders although they may occasionally be found breeding in bamboo stubs. They are basically forest

tants (Fig 33) but they do extend from forests into coffee plantation as noted by Boshell Manrique and Osorno Mesa (1944) may be ob to follow man for 200 to 300 yards into open cleared land in pursuit blood meal Furthermore it is not unusual to find these mosquitoes b ing in tree holes in parks in small towns, where they may occasionally be tured in nearby houses Nevertheless their true habitat is the forest In s



★ *Haemagogus capricornii* ● *H. spegazzinii* □ *H spegazzinii falco* ○ *H uriartei* Δ *H tropicalis*

Fig 33 Localities in Brazil where *Haemagogus* species have been captured

locations they have a marked preference for the upper levels in the forest canopy In fact it was the observation by Boshell Manrique (Bugher, Boshell Manrique et al , 1944) that mosquitoes of this genus appeared suddenly hen trees were being felled that precipitated the study of vertical distribu on which has been of inestimable importance in unraveling the epidemiol y of yellow fever The genus *Haemagogus* shows a marked preference for midday period as a feeding time This was first reported by Kumm and ra (1938) who also first mentioned that *H janthinomys* could be caught gh as 30 feet above the ground in trees With the more intensive study is genus since its recognition as a vector of yellow fever, its strict prefer for feeding during the noon hour has been repeatedly emphasized is known about the blood preferences of the genus in nature Wh

Bates and Roca Garcia (1946b) noted that it is much more difficult to in specimens to feed on marsupials in the laboratory than on monkeys may be due in part to the relative cleanliness of the laboratory anim Certainly marsupials do become infected and immunized to a certain ext in nature and it must be presumed therefore that they are bitten by m quitoes

It is of interest that two species of *Haemagogus* have been successfull colonized by Osorno Mesa in Colombia *Haemagogus equinus* (Osorno Mesa 1944) and *H splendens* (Osorno Mesa 1947)

Haemagogus spegazzinii Brethes

Antunes and Whitman (1937) first reported the laboratory transmission of yellow fever by bite of mosquitoes of the genus *Haemagogus* In their re port they classified their specimens as *H janthinomys* When subsequently Shannon Whitman and Franca (1938) isolated yellow fever virus from wild caught mosquitoes that they identified as *H capricornii* these were thought to be of the same species in that Antunes (1939) had formed the opinion that the original females used in the earlier studies corresponded with females collected in Sao Paulo the type locality of *H capricornii* How ever Cerqueira and Boshell Manrique (1946) have shown that the only spe cies of the genus *Haemagogus* encountered in the locality in which Antunes and Whitman collected their specimens is in reality *H spegazzinii* a species which was for a while considered the same as *H capricornii* and was later confused with *H equinus* Subsequently yellow fever virus was isolated from wild caught females of the species *H spegazzinii* by Laemmert de Castro Fer reira and Taylor (1946) In further laboratory studies with this species Waddell and Taylor (1945) also secured transmission by bite

This species of *Haemagogus* has perhaps the widest distribution of all members of the genus Recently Kumm and Cerqueira (1951a) have sum marized the known distribution for the important members of this genus and indicated that *H spegazzinii* occurs virtually throughout Brazil as well as into Argentina on the south and to the Island of Trinidad to the north In the northwest sector of Brazil however it is mostly replaced by the sub species *H spegazzinii falco*

Haemagogus spegazzinii falco Kumm Osorno and Boshell

Bugher Boshell Manrique et al (1944) and Boshell Manrique and Osorno Mesa (1944) reported the repeated isolation of yellow fever

lombia from wild-caught members of this species calling them *H* cap-*ricornii* Later Bates and Roca Garcia (1945 and 1946*b* and *c*) performed laboratory experiments with these mosquitoes which have been re to in earlier sections of this chapter They too used the name *H caprico* As a result of the findings of Kumm Osorno Mesa and Boshell Manr (1946) the name has been changed to *H spegazzinii falco* This specie virtually interchangeable with *H spegazzinii* as a vector of yellow fever fact Kumm and Cerqueira (1951*a*) indicate that there is some doubt abo the consideration of this as a true subspecies in that a complete series intergrades between *H spegazzinii* and *H spegazzinii falco* has been found Perhaps for the purpose of the dissemination of yellow fever there is no dif ference

H spegazzinii falco is found from the northwestern section of Brazil through Colombia into Panama Courtney (1950) and Galindo Trapido and Carpenter (1950) reported its presence in the region of the Canal Zone where yellow fever recently made its appearance

Haemagogus capricornii Lutz

Shannon Whitman and Franca (1938) reported the isolation of yellow fever virus from specimens of *H capricornii* captured in an area north of Rio de Janeiro in which jungle yellow fever was active at that time Subsequent investigations of the *Haemagogus* population of that area reported by Waddell and Kumm (1948) showed that while *H capricornii* was perhaps the predominant species *H spegazzinii* also occurred While it is generally believed that Shannon s identification was probably correct Waddell and Kumm (1948) have performed laboratory transmissions of yellow fever by the bite of fully identified *H capricornii* which indicate that for practical purposes this species is equivalent to those previously discussed as vectors of yellow fever

In distribution this species is more southerly than those previously de ribed While reported from Bahia Minas Geraes and Espirito Santo razil it is more common in the State of São Paulo and south to Rio Grande Sul

emagogus equinus Theobald

ddell and Taylor (1945) successfully used *H equinus* as vector in a y of the cyclic passage of yellow fever virus through marmosets and uitoes Later these authors (Waddell and Taylor 1947) compared th

species of *Haemagogus* with *A. aegypti* as vectors of yellow fever in the laboratory. In their studies roughly twice as many individuals of the latter species as of the former became infected on the same blood meals. However, as pointed out earlier, this may not be a true representation of the capability of *H. equinus* to become infected and transmit virus in nature. Furthermore, the specimens used by these authors were from a laboratory-reared stock originally colonized by Osorno-Mesa in Colombia, which admittedly did not produce adults that survived long. Perhaps the relative efficiency of *H. equinus* would have been higher had wild-caught specimens been used.

H. equinus Theobald is to be found in the north and west portions of South America from Bolivia and western Colombia to Panama. In a recent survey by Galindo, Trapido, and Carpenter (1950) of the day-flying mosquitoes in the sections of Panama adjacent to the Canal Zone, it was the most abundant species of *Haemagogus* encountered. From the data presented by these workers, it is obvious that while *H. equinus* is predominantly arboreal, it is perhaps not as restricted to the higher levels of the forest canopy as is *H. spegazzinii falco*.

Haemagogus splendens Williston

Anderson and Osorno-Mesa (1946) first studied the ability of *H. splendens* to transmit yellow fever by bite. Using the night monkey *Aotus trivirgatus* as the susceptible vertebrate, they had no difficulty in passaging the virus through two *H. splendens* cycles and back to *A. trivirgatus*. In these studies they demonstrated an incubation period of 14 to 16 days at 30°C. Their findings regarding the amount of virus required to infect the mosquitoes have already been discussed. Waddell (1949) has likewise found that *H. splendens* will transmit the virus, though the efficiency of the species in comparison with *A. aegypti* was low. However, Waddell was using laboratory-bred specimens that may not have been as satisfactory hosts for the virus as specimens occurring in nature.

H. splendens was originally described from St. Vincent, British West Indies, and later was shown to be present in Venezuela. In Colombia it has been found in the northern coastal section and in the llanos with some frequency. It was not recorded by Galindo, Trapido, and Carpenter (1950) in their recent survey in Panama.

Haemagogus uriartei Shannon and Del Ponte

Little work has been done with this species of *Haemagogus* Res studies by Antunes and Whitman (1937) indicated that like other s of the genus *H uriartei* is susceptible to infection with yellow fever However the only mosquito surviving for 16 days failed to transmi virus by bite although virus was present in its body The mosquitoes in these studies were found by Antunes breeding in tree holes in back y in the town of Esplanada Bahia

H uriartei has a somewhat restricted distribution in Brazil being fou mostly in the northeast from the State of Bahia to Pernambuco Howeve it has been recorded from the States of Goiás and Mato Grosso

Haemagogus tropicalis Cerqueira and Antunes

This species of *Haemagogus* was found by Kumm and Novis (1938) at Curralinho Pará Brazil on Marajo Island in the mouth of the Amazon River No information is available regarding its geographic distribution or capability of transmitting yellow fever Kumm believes that it does not feed on man

Other species of *Haemagogus*

Species of *Haemagogus* from Colombia concerning which there is little information are

Haemagogus anastasionis Dyar (also reported from Panama Costa Rica and El Salvador)

Haemagogus andinus Osorno

Haemagogus boshelli Osorno

Haemagogus chalcospilans Dyar (also reported from Panama and Costa Rica) Galindo Trapido and Carpenter (1950) stated that in Panama this species was captured only in the coastal mangrove swamps and never in the forest

Haemagogus lucifer Howard Dyar and Knab (also reported from Panama and Costa Rica) Galindo Trapido and Carpenter (1950) reported this species to be very common in the forests in the neighborhood of the Canal Zone While primarily arboreal a greater percentage was caught at ground level than was the case with either *H equinus* or *H spegazzinii falco*

Species collected in Costa Rica and Salvador Central America are

Haemagogus iridicolor
Haemagogus mesodentatus
Haemagogus regalis

It is obvious that the genus *Haemagogus* extends well into Central America Should the last mentioned species be as capable of transmitting yellow fever as those already investigated at least part of the requisites for the maintenance or spread of yellow fever in that area exists

Tribe *Sabethini*

The sabethine tribe of mosquitoes is of interest because of the fact that Shannon Whitman and Franca (1938) isolated the virus of yellow fever from the bodies of a mixed lot of 88 specimens of the genera *Sabethoides* *Limatus Wyeomyia* and *Trichoprosopon* Not only was it impossible to determine which genus contained the virus but it was not known whether the mosquito (or mosquitoes) harboring it was truly infected or had recently fed on blood that contained virus Nevertheless this precipitated a considerable amount of laboratory investigation of which unfortunately little has been published

One of the chief difficulties encountered in the investigation of *Sabethini* has been the high death rate of specimens brought to the laboratory Various methods of keeping them alive have been tried without much improvement in survival time During the course of certain partially successful experiments carried out with *T frontosus* to be discussed below a technique was adopted whereby a large porous earthenware candle was filled with water and placed in the cage The mosquitoes attracted apparently by the damp microclimate roosted on the candle This kept them alive for a somewhat longer period of time but at what temperature is not certain Obviously with the excessive evaporation off the candle surface the microclimate must have been cooler than the average cage temperature Certainly the artificial environment supplied to the mosquitoes must have been far from the optimum environment previously discussed

With regard to nomenclature this predominant tribe of tropical American mosquitoes has undergone various taxonomic revisions As a result species names and even generic names have been changed During 1910 to 1911

Lane and Cerqueira (1912) undertook a revision of the tribe It is their classification that will be followed in the brief discussion below

Genus *Sabethes* subgenera *Sabethes, Sabethoides, Sabethinus*

In general, the various species included in this genus are similar to species of the genus *Haemagogus* in being arboreal, day flying insects They are mostly tree hole or bamboo breeders Although their distribution may indicate a theoretical relation to the transmission of yellow fever, the results of a limited number of laboratory studies suggest that they are not susceptible to the virus

Genus *Phoniomyia*

What knowledge we have of the breeding habits of these delicate sabethines indicates that they deposit their eggs chiefly in the water in the leaf bases or in the central water container of epiphytic plants They are arboreal and day flying, though without the marked midday activity of mosquitoes of the genus *Haemagogus* Laboratory studies have failed to show that they can transmit yellow fever, but it has been obvious that the usual cage conditions are unsatisfactory for them

Genus *Wyeomyia* subgenera *Wyeomyia, Nunezia, Cruzmyia, Davismyia, Menolepis, Antunesmyia, Dendromyia*

While scattered laboratory studies on several species of the genus *Wyeomyia* have indicated that these mosquitoes are not infectible with yellow fever virus by routine methods, Bugher was able on one occasion to transmit yellow fever by the bite of two specimens tentatively identified as *Wyeomyia melanocephala*, which had been immersed in the larval stage in a concentrated virus suspension Unfortunately, so little is known of larval infections that it is impossible to state whether or not these results are significant as far as indicating the true susceptibility of the species Needless to say, the amount of study as yet accorded this genus has not been sufficient to warrant eliminating it from further consideration

The genus has a wide distribution in the Americas, one species even being found in the northern United States Its breeding places of choice are predominantly plant axils, epiphytic plants, and bamboo stubs It is day flying but without predilection for the noon hours Certain species that breed in colocasia may be considered to be peridomestic

Genus *Limatus*

Such studies as have been made on the genus *Limatus* have indicated th[at] it is not susceptible to yellow fever. It is of interest, however, that the com[mon]est species, *Limatus durhami*, is peridomestic and often breeds in such artificial containers as tin cans and bottles around houses.

Genus *Trichoprosopon*, subgenera *Trichoprosopon, Limamyia, Ron[...] plessenia, Shannoniana, Isogoeldia, Ctenogoeldia, Hyloconops*

Of the sabethines the genus *Trichoprosopon* is the most provocative in that laboratory studies on one species, *T. frontosus*, indicate that it can transmit yellow fever by bite (see below). The genus is widespread throughout tropical America. Many of the species breed in plants, either in the axils or in the central water containers. Some breed in fallen nutshells or in water stored in fallen leaves. Still others breed in bamboo. *T. frontosus* is apparently widely distributed in eastern Brazil and extends up to British Guiana and Trinidad. It is day flying and forest inhabiting and as a rule is caught at ground level. It has a tendency to maintain a significant density at all seasons of the year. Plants of the type in which it breeds, unlike many tree holes and ground pools, are capable of retaining enough water over most of the year to permit a continuous supply of adults. D. F. Davis (1945) discussed the relative seasonal prevalence of mosquitoes of this genus in an intensive study of two small Brazilian forests.

Whitman, Tulloch, and Waddell found that specimens of *T. frontosus* retained the virus in their bodies for long periods of time, although in low concentrations. At a later time, with higher laboratory temperatures, they obtained transmission by bite on one occasion (Waddell, 1949). Subsequent studies by Waddell confirmed the transmission by bite. However, it must be pointed out that the effectiveness of this species in the laboratory was greatly inferior to that of such vectors as *A. leucocelaenus* or the several species of *Haemagogus* investigated. Whether this relative ineffectiveness is due to the abnormal environment supplied these mosquitoes in the laboratory or whether they are inefficient vectors is unknown.

The genus *Trichoprosopon* now includes the several species originally in[c]luded in the genus *Goeldia*. These latter species have been given the sub[ge]neric name *Hyloconops*. It is to this subgenus that *T. frontosus* belongs. [Th]e genus itself is interesting in that, as Lane and Cerqueira state, [...]

easily separated from the rest of the sabethines on anatomical grounds being the group closest to the tribe *Culicini*

Genus *Culex*

Transmission experiments with species of the *Culex* genus in South America have yielded in the main negative results In fact in many cases yellow fever virus rapidly disappeared from these mosquitoes and could not be demonstrated by inoculating extracts of their bodies into susceptible animals Yet occasionally specimens were found to transmit the virus by bite Perhaps the experiments reported by N C Davis (1933*b*) with *Culex fatigans* are the most suggestive In this series two transmissions by bite were obtained after incubation periods of 17 and 20 to 23 days Mosquitoes of the lot transmitting by bite after 20 to 23 days were shown to have virus in their bodies when injected into monkeys on the 24th day but 16 mosquitoes of the lot transmitting by bite on the 17th day were ground up and injected into a monkey 29 days later without infecting it In other lots of *C fatigans* virus could be detected in the bodies on the 2d day after their infecting meal but not on the 4th or subsequent days This variability is much more marked than any recorded in studies on the genera *Aedes Taeniorhynchus* and *Psorophora* or even *T frontosus* Davis also tested *Culex nigripalpus* as reported by Whitman and Antunes (1937) for its capacity to transmit yellow fever In these studies virus was never transmitted by bite and was recoverable by inoculation of the ground up mosquitoes on only two of 15 occasions

In general the consensus of opinion is that the genus *Culex* has little to do with the transmission of yellow fever in nature

IMPORTANT SPECIES OF AFRICAN MOSQUITOES

In Table 8 are listed the recorded laboratory studies on African mosquitoes While the list is shorter than that of South American mosquitoes given in Table 7 the number of species found capable of experimental transmission is as great It is to be noted that many of the experimental vectors are *Aedes* of the subgenus *Stegomyia* which is represented in South America by *A aegypti* only In contrast to findings in South America one species of *Taeniorhynchus* and a species of *Culex* in Africa have been shown to transmit the virus by bite

TABLE 8

SPECIES OF AFRICAN MOSQUITOES STUDIED IN THE LABORATORY

Mosquito species	*References*	*Results*
Anopheles gambiae	Philip 1930*a*	N
Taeniorhynchus africanus	Philip 1930*a*	T
Taeniorhynchus uniformis	Kerr 1932	S
Aedes aegypti	United States 61st Congress Yellow Fever 1911	T *
Aedes africanus	Philip 1929*a*	T *
Aedes apicoargenteus	Bauer 1928	N
Aedes luteocephalus	Kitchen Haddow Smithburn (unpublished)	S
Aedes metallicus	Bauer 1928	T
Aedes simpsoni	Lewis Hughes Mahaffy 1942	T
Aedes vittatus	Philip 1929*a*	T *
Aedes cumminsi	Philip 1929*a*	T
Aedes irritans	Kitchen Haddow Smithburn (unpublished)	S
Aedes nigricephalus	Philip 1930*b*	S
Aedes stokesi (= *apicoannulatus*)	Philip 1930*b*	S
Aedes lineatopennis	Bauer 1928	T
Aedes punctocostalis	Kerr 1933	S
Aedes taylori	Philip 1930*b*	S
Aedes grahami	Lewis Hughes Mahaffy 1942	T
Eretmapodites chrysogaster	Kitchen Haddow Smithburn (unpublished)	N
Culex thalassius	Bauer 1928	T
	Kerr 1932	T

N = not susceptible S = susceptible T = transmits by bite
* Found infected in nature

Genus *Aedes*

Aedes (Stegomyia) aegypti Linnaeus

Great interest attaches to the fact that the ecology of *A aegypti* is not con-
stant for the species in its various geographic habitats In South America
it is an intensely domestic species rarely captured at any distance from hu-
man habitation and its peridomesticity sets it off markedly from other mem-
bers of the genus This behavior pattern is true for the species not only in
South America but in all the Americas and throughout large parts of its
essentially worldwide range In contrast *A aegypti* in certain parts of
East Africa has such a divergent ecology that it is not only of no sign

in the transmission of yellow fever but it can only with diffic tured with human but In Africa therefore this species must be is having a variable ecology dependent on locality

In West Africa *A aegypti* is essentially domestic, as it is in South However Dunn (1927*a* and *c*) in a series of studies on this spec environs of Lagos in southern Nigeria noted that it would breed in or natural containers further away from human habitations than i South America Nevertheless studies of Beeuwkes Kerr et al (193 distribution and survival time showed that the extent of its domestic ing and its longevity are adequate to account for human yellow fever th out the area Moreover virus was isolated from the species by Beeuwke Hayne (1931) and in more recent times by Bugher In fact Bugher is o opinion that all human yellow fever in Nigeria during the past 10 years best be explained on the basis of a man—*A aegypti* cycle Although ot potential vectors (some of them proved vectors in East Africa) were fou in Nigeria and were intensively investigated no evidence could be obtain of their participation in the yellow fever epidemics during this time

It was therefore of interest to find that in Bwamba County Uganda Eas Africa *A aegypti* although present was so different in its life habits as to make it virtually incapable of aiding in the spread of yellow fever Haddow (1945*a*) gave by far the best summary of this situation In his studies in the Bwamba area he found *A aegypti* breeding in tree holes rather than in artificial containers In an examination of 122 tree hole foci *A aegypti* made up 6 per cent of the larvae recovered and was fifth in abundance of the 21 species identified In contrast in only two of 31 artificial water containers in and around huts were any larvae found and in only one were *A aegypti* larvae present Haddow also quoted studies of Harper in the same area in the course of which this species was encountered breeding in only five of 569 huts (138 huts had larvae of other species of mosquitoes) while of 157 tree holes examined 17 contained larvae of *A aegypti* Similar findings have been reported by other investigators in East Africa (Wiseman Symes et al 1939 Teesdale 1941) Garnham Harper and Highton (1946) in an investigation of the Kaimosi forest in Kenya Colony reported that in an examination of 85 huts in May 1943 *A aegypti* larvae were encountered but once and in June 1944 a search of 100 huts failed to reveal any At the same time they found the species breeding in holes in recently felled trees and in rock pools in river beds These larval foci were frequently one or more miles

human habitation in the forest. During their investigation not one adult *A. aegypti* was taken in huts and the species formed only a small proportion of the total adult catches in the open. Failure to capture *A. aegypti* in the Bwamba area was in particular stressed by Haddow. Among the 50,000 mosquitoes that he examined during the first year and one half of his studies only five *A. aegypti* were identified. Mahaffy, Smithburn et al. (1942) reported the capture of but one specimen during extensive catches in Bwamba. Haddow summarized his findings with the statement: "The inevitable conclusion is that in Bwamba *A. aegypti* is mainly a sylvan species and must be essentially zoophilic."

In the Nuba Mountain region Lewis (1943) found *A. aegypti* to be somewhat variable. In certain areas these mosquitoes could be seen in almost every house inspected, in others only one house in 20 contained them, and in still others the species was absent. Lewis recorded that while *A. aegypti* larvae were observed on 26 occasions in domestic breeding places, they were also found on 21 occasions in tree holes and on seven occasions in rock pools.

This variation in domesticity of *A. aegypti* suggests that perhaps the original population was essentially sylvan but with a strain tendency to domestication. During years of east to west migration the offspring of the more domesticated individuals may have been carried in water receptacles and become established in West Africa. From here further transport might again select the most domestic strains for introduction into the Americas. Possibly the route of transportation lay north through Egypt to the Mediterranean. Certainly their domestic behavior as eventually found in the regions removed from East Africa must have been the result of strain selection.

Before leaving the African *A. aegypti* it is worth recording that as far as is known all strains of this species can transmit yellow fever virus. Dinger, Schuffner et al. (1929) and Hindle (1930) were able to transmit the virus with specimens from Java, and Lewis, Hughes and Mahaffy (1942) found that a pale variant from the Red Sea area likewise transmitted readily. However, despite this evidence there is still room to question the relative vector efficiency of *A. aegypti* from different geographic areas. In fact, the failure of yellow fever to extend to India and the Orient might possibly be due to a barrier composed of *A. aegypti* with a reduced susceptibility to the virus. Unfortunately, to verify this supposition would require the testing of large numbers of specimens reared with the most rigid precautions to prevent cross breeding of the races under study.

Aedes (Stegomyia) africanus Theobald
Aedes (Stegomyia) luteocephalus Newstead
Aedes (Stegomyia) pseudoafricanus Chwatt

These three species have been grouped for the purpose of this
because in the first place *A pseudoafricanus* has only recently be
entiated from *A africanus* At the time of the original investigations
canus by Philip (1929*a*) this separation had not been made and it is
possible to say with which species he worked Secondly although *A a*
and *A luteocephalus* have long been considered as separate species
and Mahaffy (1949) found evidence that would indicate that they may
sent the extreme forms of variable species Under the circumstance
preferable to deal with them as a unit pointing out such differences in
ogy as have been recorded

Bauer (1928) was the first to study the capacity of *A luteocephalus* to t
mit yellow fever by bite He found that mosquitoes of this species rea
transmitted the virus and that the bite of one female 53 days after its inf
ing meal was sufficient to cause rapidly fatal yellow fever in an experimen
rhesus monkey Philip (1929*a*) showed that *A africanus* could likewise tra
mit the virus by bite Thus the potential importance of these two specie
was established at the beginning of the modern era of yellow fever investi
gation Following the separation of *A pseudoafricanus* from *A africanus* by
Chwatt (1949) experiments with the new species indicated that it also could
transmit yellow fever by bite (Bruce Chwatt 1950)

Dunn in studies in the Lagos area Nigeria (1926 and 1927*b*) found *A*
luteocephalus to be the commonest species breeding in tree holes with *A*
africanus in third or fourth place Philip (1933) confirmed this Kerr (1933)
reporting on the distribution and habits of West African mosquitoes noted
the extreme rarity with which specimens of either *A africanus* or *A luteo*
cephalus were taken in houses in contrast to their abundance outdoors He
pointed out that the principal feeding time of these two species is at twilight
(mostly between the hours of 7 and 8 P M) and that they bite man with
great frequency But perhaps the most complete information concerning the
habits of *A africanus* has been compiled by Haddow and his associates (Had
dow Gillett and Highton 1947 Haddow and Mahaffy 1949) in studies
in Bwamba County Uganda In the first place they confirmed Kerr s ob
servations on the optimal feeding time Secondly they showed that *A afri*
canus is primarily arboreal and that it is captured in max

50 to 60 ft above the ground At such altitudes it may be caught at all sons of the year With regard to choice of food Haddow and Dick (19 have indicated that *A africanus* has a preference for monkey blood ov human blood and concluded that this species feeds mainly on monkeys i nature Mattingly (1949) has reported studies on the vertical distribution and biting cycle of *A africanus* in the Lagos area of Nigeria with findings essentially the same as those of Haddow and his associates However it is to be noted that in contrast to Dunn Philip and Kerr Mattingly failed to cap ture *A luteocephalus* This failure however was not due to a recent dis appearance of this mosquito from southern Nigeria but merely reflects the fact that in the Lagos area different forest patches have a somewhat different species makeup Thus the absence of *A luteocephalus* at Mattingly s station is of significance only as far as that individual forest station is concerned A W Taylor (1934) noted that *A luteocephalus* was common in northern Nigeria while *A africanus* was common in southern Nigeria and was not present in the north

With regard to the ecology of the three species under consideration *A luteocephalus* and *A africanus* are so similar as to be frequently discussed together However Haddow and Mahaffy (1949) in their discussion of the relationship between the two species said though overlap occurs *A luteocephalus* is found in large tracts of fairly dry and open country where the essentially sylvan *A africanus* is not known to be present If such an ecologic difference is widespread it is obvious that *A luteocephalus* may eventually be found to be of significance in the dissemination of yellow fever in areas in which *A africanus* is less well established In contrast to the markedly similar altitude preferences of those two species *A pseudo africanus* is apparently less prone to an arboreal existence Following as yet limited observations Bruce Chwatt (1950) reported that 70 per cent of 403 specimens of *A pseudoafricanus* were captured at ground level and only 30 per cent at the 30 foot platform Haddow, Gillett and Highton (1947) and Mattingly (1949) on the other hand captured at ground level only 1 to 7 per cent of their total *A africanus* catch Furthermore *A pseudoafri canus* has as yet been found breeding only in tree holes in the white man groves in the Lagos area and thus may have a restricted distribution

Turning now to the findings that have proved that *A africanus* does transmit yellow fever in nature the first of these was the isolation by Smith rn and Haddow (1946) of yellow fever virus from the bodies of a mixed up of 12 species of *Aedes* captured at Mongiro Bwamba County

ile theoretically any of the 12 species might have contained the virus authors felt that the most suspect species in the group was *A africanus* rogram of placing susceptible monkeys in cages in the treetops was there instituted in the hope that wild mosquitoes would infect them thus icating the presence of virus in the area In association with this pro m Haddow Smithburn et al (1948) infected a rhesus monkey by in ion of specimens of wild *A africanus* captured in the forest canopy near sentinel monkeys However at this time they found that *A africanus* ild not enter a cage to feed on monkeys despite its preference for mon blood The sentinel monkeys were therefore removed from their cages loosely chained to their platforms Within 6 months of this time Smith n Haddow and Lumsden (1949) found evidence of yellow fever in these ikeys and an intensive capture of mosquitoes for virus isolation was un taken During this period a total of 2 883 specimens of *Taeniorhynchus* ies 1 329 specimens of tree *Aedes* species and 22 specimens of *A simp* were identified and inoculated into rhesus monkeys without evidence heir harboring virus In contrast virus was isolated four times from the ies of *A africanus* (2 010 specimens) by monkey injection and from one of 91 specimens by bite This definitely proved that *A africanus* was ying a significant role in the dissemination of yellow fever in the forest

he distribution of *A pseudoafricanus* is as yet undetermined but should oreeding be restricted to tree holes in mangroves it would presumably a coastal species only

he distribution of *A africanus* and *A luteocephalus* on the other hand idespread throughout tropical Africa extending from West Africa across ast Africa and from the Anglo Egyptian Sudan (Lewis 1943) (*A luteo halus*) as far south as Bechuanaland (de Meillon 1946) (*A luteocepha* It is of interest that de Meillon states that *A luteocephalus* was the y mosquito captured while biting man at 50 ft above the ground

les (Stegomyia) simpsoni Theobald

hilip (1929a) first showed that *A simpsoni* could transmit yellow fever is by bite thus establishing the potential importance of the species owing to the scarcity of this mosquito in West Africa it was not at that e considered very important in the epidemiology of yellow fever Studies Dunn (1926 and 1927*b*) Philip (1933) and Kerr (1933) indicated that the ies could be found breeding in tree holes but not in sufficient numbers oe of interest In contrast to these results Haddow (1945*b* and *c*) has

found *A simpsoni* to be a very common species in the banana and coloc plantations in and around the human habitations in Bwamba Coun Uganda In this situation it breeds profusely in the plant axils rather th in tree holes In detailed studies Haddow showed that this mosquito whi taking blood from birds hens goats and monkeys in decreasing order pre ferred human blood In studies on the time and place of feeding he noted that the species was strictly diurnal feeding most readily in the thinner parts of the plantations where young plants predominate and around the edges It did not enter the houses but did penetrate the extreme edges of the rain forest Studies on preferred altitude showed that although more mosquitoes were caught at ground level than at 18 ft above ground the difference was not great In further studies Haddow (1948) was able to ob tain larvae of this species from plant axils during every month of the year indicating an appreciable and constant density at all seasons

Interest in *A simpsoni* stems chiefly from the isolation in 1941 of yellow fever virus from two lots of wild caught specimens by Mahaffy Smithburn et al (1942) These isolations occurred during a time when virus was present in the human population Owing to the prevalence of this species and the absence of *A aegypti* all evidence pointed to an epidemic of the urban type with *A simpsoni* replacing *A aegypti* as the vector Subse quently a vaccination campaign was undertaken in this region with excellent results Nevertheless in 1942 in the absence of any evidence of human yellow fever virus was isolated from another lot of *A simpsoni* (Smithburn and Haddow 1946) This finding favors the belief that *A simpsoni* not only can act as a vector of urban yellow fever but can become infected by feeding on sources of virus other than man

A simpsoni has a wide distribution throughout Africa extending from Sierra Leone on the west to Dar es Salaam in Tanganyika on the east and from the Anglo Egyptian Sudan on the north to Natal in the south Within this area the selection of favored breeding sites is not constant For example is has been stated *A simpsoni* is a tree hole breeder in many parts of its range such as Nigeria In contrast in Bwamba it has adapted itself strictly o breeding in plant axils particularly the peridomestic banana and colo asia There is reason to believe that the changing agricultural customs of he native African populations are having a significant effect on the impor nce of this species For example the introduction of rice as a principal p into the Bwamba area may reduce banana culture and thus reduce the ored sites of *A simpsoni* breeding On the other hand there

recent extension of banana planting in or near native houses in the Cameroons with a consequent increase in the population of *A simpsoni* While there has as yet been no evidence in this area of transmission of yellow fever by *A simpsoni* it is natural to conclude that sooner or later such transmission will take place

Aedes (Stegomyia) vittatus Bigot
Aedes (Stegomyia) metallicus Edwards
Aedes (Diceromyia) taylori Edwards
Aedes (Diceromyia) furcifer Edwards

The grouping of the four species above is based on the fact that one or more of them was suspected of having played a leading role in the yellow fever epidemic occurring in the Nuba Mountains of the Anglo Egyptian Sudan in 1939 The most important of them is perhaps *A vittatus* This mosquito was first shown by Philip (1929*a*) to be readily capable of transmitting yellow fever virus in the laboratory by bite He found it to be a rapidly maturing and semidomestic species favoring rain holding depressions in rocks and masonry for purposes of breeding Kumm (1931*b*) noted that aside from breeding in rock holes members of the species were often observed in Kano Nigeria breeding in water pots Lewis (1943) reporting on his survey of the mosquitoes of the yellow fever area in the Nuba Mountains stated that *A vittatus* was probably the most abundant biting mosquito in the rains It was not only relatively common in evening catches but was the only common day biting species out of doors It was sometimes found breeding indoors in water vessels Evidence given by Kerr (1933) and Lewis (1943) indicates that this species is primarily crepuscular feeding mainly in the first hour after sunset It is of interest that the species has an unusually wide geographic distribution Not only is it widely dispersed throughout Africa but it has been collected in Corsica (original description) Arabia India and eastward as far as French Indochina

A metallicus was first shown to be capable of transmitting yellow fever by bite in the laboratory by Lewis Hughes and Mahaffy (1942) It is a tree hole breeder found by Lewis (1943) to be one of the more frequent biters of man in the Nuba Mountains in sites near its breeding foci However it was infrequently taken at any distance from such foci Although the species has a fairly extensive distribution throughout Africa it has not been found outside the Nuba Mountain area in sufficient density in association with yellow fever to incriminate it as a significant vector in nature

ri and *A furcifer* are so closely related as to make the separation
cies by the examination of females at times difficult Lewis (1943)
t of his entomologic survey of the Nuba Mountains preferred to
em as a group The only laboratory studies on their capacity to
ellow fever were made by Lewis Hughes and Mahaffy (1942)
ylori which transmitted the virus readily by bite Both species are
breeders but in the Nuba Mountains they were found biting man
ly further away from their breeding sites than was *A metallicus*
ng to F W Edwards (1941) *A furcifer* has a wide distribution
orded in West Africa the Sudan and south through Uganda to
ansvaal In contrast *A taylori* is recorded only from Nigeria and
a It is probable however that the distribution of the latter species
tensive than indicated Aside from the potential role of these two
the Nuba Mountains they have not been considered important
ion with yellow fever outbreaks in other parts of Africa

dimorphus) stokesi Evans (*apicoannulatus* Edwards)

1928) showed that *A stokesi* identified in his report as *A apicoan*
ould transmit yellow fever by bite thus establishing it as a poten
r However there has been little subsequent information that
id to mark this species as an active participant in the dispersion
fever Kerr (1933) reported that although the larvae of *A stokesi*
monly found in tree holes in the Lagos area he failed to obtain
dult specimen in catches inside human habitations Furthermore
uito was rarely caught outside on human bait Kerr was of the
hat these observations indicated a food preference other than
ood

ecies has been captured most frequently in West Africa from Sierra
Nigeria However Kumm (1931*a*) noted records of its presence in
vaal and Edwards (1941) recorded it from Uganda

ynchus

ynchus (Mansonioides) africanus

1930*a*) transmitted yellow fever virus by the bite of *T africanus*
es of mosquito is a serious pest in that it attacks man readily even
the houses It is a night feeder rather than a crepuscular one
Gillett and Highton (1947) reported that in Bwamba County

Uganda *T africanus* is frequently captured at the high platform (50 ft) level In a later report Haddow and Mahaffy (1949) gave figures to indicate that next to *A africanus* and *Anopheles gambiae, T africanus* is the most common arboreal mosquito in the forest canopy in the evening and early night While Mattingly (1949) in his observations in the Lagos area did not find as great a density of *T africanus* at 50 ft there was nevertheless an appreciable number of them caught at 10 ft With regard to their sea sonal distribution these mosquitoes whose larvae attach themselves to the rootlets of aquatic plants for respiration have a somewhat unusual seasonal curve tending to peak during the early part of the dry season at a time when most of the other mosquitoes are diminishing in number

Despite their prevalence their avidity for biting humans and their ca pacity to invade the treetops no actual evidence of their playing a part in the transmission of yellow fever in nature has been obtained In fact during the period that Smithburn Haddow and Lumsden (1949) isolated yellow fever virus from four lots of *A africanus* no virus was isolated from an equivalent total number of *Taeniorhynchus* spp captured in the same locality at the same time The species is found throughout Africa south of the Sahara desert except in the more southerly portion of the Union of South Africa

Eretmapodites

Eretmapodites chrysogaster Graham group

Interest in this group of species is based on the demonstration by Bauer (1928) that *E chrysogaster* could transmit yellow fever virus by bite Subse quently it has been found that there are several closely allied species of the genus *Eretmapodites* that are not easily separable one from the other and it is therefore not known which particular species Bauer used for his experi ments

Mosquitoes of this group are widespread throughout Africa south of the Sahara desert They are restricted to forests or to regions of dense vege tation such as banana plantations According to Haddow (1945*b*, 1946) they bite during the daytime particularly in the afternoon and are rarely taken at night They are seldom captured at any distance above the ground Although the virus of yellow fever has never been isolated from wild caught specimens Smithburn Haddow and Gillett (1948) have recovered the virus of Rift Valley fever from wild caught specimens on three different occasions

Culex

Culex thalassius Theobald

This is the only species of *Culex* in Africa shown to be capable of transmitting yellow fever by bite. However Kerr (1932) was able to obtain transmission only after long periods of incubation. Such prolonged incubation, needless to say, would reduce the theoretical efficiency of the species as a vector. Furthermore, although this species is common along the coastal fringe of Africa both west and east, it is absent in the interior and probably has little contact with the virus of yellow fever in nature.

ISOLATION OF OTHER VIRUSES FROM WILD CAUGHT MOSQUITOES

Before leaving the subject of the relationship between mosquitoes and viruses it is worth while noting that in South America several unknown viruses capable of infecting white mice when inoculated intracerebrally have been isolated from wild caught mosquitoes. In Colombia, Roca Garcia (1944) has described three such viruses, two isolated from *Anopheles (Kertes...ia) boliviensis* and one from *Wyeomyia (Dendromyia) melanocephala*. In Brazil, Laemmert and Hughes (1947) isolated a virus from a pool of mixed *Aedes* and *Psorophora* caught in Ilheus. The predominant species were *A. serratus* and *P. ferox*, both of which were shown to be capable of transmitting the virus by bite in laboratory experiments. *A. aegypti* was also found to be a vector of this virus in the laboratory. More recently viruses have been isolated from *Sabethes* and from *Haemagogus* and *A. leucocelaenus* near Passos, Minas Geraes. Early studies on these by Hughes and Perlowagora (1950a) indicated that most of them are similar if not identical to one another, though probably unrelated to the Ilheus virus.

Similar isolations of hitherto unknown viruses have been made in equivalent studies in Africa. In Uganda, Smithburn and Haddow (1944) isolated from the *Aedes (Aedimorphus) abnormalis* Theobald group a previously unknown virus capable of infecting mice on intracerebral inoculation. This virus was given the name Semliki Forest virus.

Later Smithburn, Haddow and Mahaffy (1946) reported the isolation of a second unknown virus which they called Bunyamwera virus from its place of origin. This virus was recovered from a large group of over 1,000 s...

mens including 14 species in five subgenera of *Aedes* A third virus called Mengo virus was recovered from wild caught mosquitoes by Dick Smithburn, and Haddow (1948) In one instance the recovery was from one species only, *Taeniorhynchus (Coquillettidia) fuscopenatus* Theobald In the second instance the virus was recovered from a mixed group of *Taeniorhynchus* species in which the above species was predominant

As previously stated, Smithburn, Haddow, and Gillett (1948) isolated the virus of Rift Valley fever from the *E chrysogaster* group on three different occasions They also isolated the same virus twice from *Aedes (Aedimorphus) tarsalis*, Newstead group, and once from *Aedes (Stegomyia) de boerri* sub species *de meilloni* Edwards

Aside from the isolations mentioned above, which have been reported in journals, three more hitherto unknown viruses have been isolated in Uganda and are currently under study The first of these Ntaya virus, was isolated in 1943 by Smithburn and Haddow from a lot of 1,318 mosquitoes including at least 13 species of the genera *Uranotaenia, Theobaldia, Taeniorhynchus, Aedes*, and *Culex* The second, Uganda S , was isolated in 1947 by Dick and Haddow from a group of 65 *Aedes* mosquitoes of the subgenera *Finlaya* and *Aedimorphus* The third, Zika virus, was isolated in 1948 by Kitchen and Haddow from a lot of *A africanus* caught near Entebbe A few months previously the same virus had been isolated by Dick from a normal rhesus monkey during an access of fever occurring while the animal was stationed as a sentinel in the canopy of the forest near where the mos quitoes that later yielded virus were caught Each of these 6 viruses isolated in Uganda (omitting Rift Valley fever virus) is a distinct entity and appar ently different from hitherto known viruses, with one exception The Mengo virus was found by Dick (1949) and Warren Smadel, and Russ (1949) to be identical with the viruses of the encephalomyocarditis group

Aside from these Uganda viruses Bugher, Macnamara and Hahn isolated a virus from *Eretmapodites grahami* captured at Kumba in the British Cameroons This virus surprisingly, has been found by Smithburn to be closely related to or identical with the Semliki Forest virus mentioned above

ARTHROPODS OTHER THAN MOSQUITOES

As was stated at the beginning of this chapter, mosquitoes have been the most actively investigated potential vectors of yellow fever However, as

soon as a susceptible experimental animal was discovered sporadic ... with other arthropods were made with varying success

Cimex (bedbugs)

Lemos Monteiro (1929) reported that the feces of bedbugs (specie... stated) could be shown to contain yellow fever virus for as long as 15 ... after they had taken a blood meal on an infected monkey However Ku... and Frobisher (1932) in a series of experiments with *Cimex hemipterus* c... cluded that the virus was recoverable for only 2 days either in the body ... in the feces and that by the 3d day the virus had disappeared Philip (1930... attempted to transmit yellow fever by interrupted feedings with *Cime*... *lectularius* but did not succeed

Triatomata (assassin bugs)

Davis and Shannon (1931a) in preliminary studies on the capability of *Triatoma megista* to transmit yellow fever were able to show that virus could survive in the larvae for a period of 7 days A single attempt to transmit by bite or inoculation at 48 days failed In further experiments N C Davis (1933a) found that specimens of *Triatoma* would retain virus in their bodies for at least 6 days and perhaps for as long as 10 days but he failed to get transmission by bite or by the inoculation of feces In a series of six interrupted feeding experiments Davis was able to transmit the virus on one occasion only This incidentally is one of the rare reports of successful transmission of yellow fever by interrupted feeding

Ctenocephalides canis (dog fleas)

Hoskins (1934b) in studies on dog fleas demonstrated that yellow fever virus would survive up to 7 hours in their bodies but could not be recovered after 18 hours In four interrupted feeding experiments with these fleas he was unable to transmit yellow fever from infected to normal monkeys

Stomoxys calcitrans (stable fly)

Hoskins (1934b) found that stable flies retained yellow fever virus up to 42 hours but not for 18 hours or longer In attempts at interrupted feeding he was able to transmit the virus both immediately and following a period of 6 hours but not later It is presumable that in this instance the transm...

was due to regurgitation rather than to the insertion of virus con
parts

Cockroaches and other assorted insects

Findlay and MacCallum (1939) reported that viscerotropic yello virus injected into the abdomen of *Blatta germanica* (the German cock remained viable for at least 15 days. Later Findlay (1941) added to th observation that *Blatta orientalis* failed to retain the virus for as long as 2 He also reported at this time that desert locusts (*Schistocerca gregaria*) can migratory locusts (*Locusta migratoria migratoria*) and the tsets (*Glossina morsitans*) likewise failed to retain the virus for 2 days but leech (*Hirudo medicinalis*) retained it for 8 days. Findlay suggested that ingestion of an infected cockroach by a susceptible animal could presumabl result in infection but did not state how cockroaches are to become infecte under natural conditions.

Phlebotomus (sand fly)

The life of these insects in captivity is short, and there is no record of successful experiments in the laboratory to test their capacity to retain yellow fever virus much less transmit it by bite. The one observation of an incriminatory nature is that published by Smithburn, Haddow, and Lumsden (1949) in which a monkey was infected with yellow fever by the injection of wild-caught *Phlebotomus*. This finding is potentially of great significance. On the other hand, the isolated observation may prove to be of no significance in that the virus isolated may have been in the stomach of a recently fed insect and would have disappeared after a day or two.

Hippoboscidae

As part of a study on the possible connection between birds and the dissemination of yellow fever virus in nature, de Castro Ferreira investigated the susceptibility of the pupiparous fly *Pseudolynchia canariensis* from domestic pigeons. They were found to be insusceptible to the virus.

Acarinae (ticks)

Perhaps the most provocative studies have been done with ticks. Aragão (1933b) reported that he was able to transmit yellow fever by the bite of *Amblyomma cajennense* and later also claimed to have demonstrated that

yellow fever virus could be passed through the egg to the next generi
These claims have not as yet been confirmed With regard to the transmis
by bite several experiments recorded by N C Davis (1933*c*) and others w
unsuccessful

Perhaps the most extensive published experiments are those of Da
(1933*c*) In these he used several species of ticks and in addition carrie
out a single experiment with chicken mites—not further identified Wit
A cajennense he was unable to get transmission by bite but he recov
ered virus by injection from one group of these ticks as late as 15 days
and possibly 28 days after their infectious meal He found that a group
of 21 adult specimens of *Argas persicus* retained virus in their bodies for 6
days In two other experiments with these ticks starting on the 9th and 10th
days after the infectious meal he recovered no virus and obtained no trans
mission by bite On several occasions he was able to demonstrate virus in
the bodies of *Rhipicephalus sanguineus* 10 15 and even 23 days after an
infectious meal but he failed to get transmission in feeding experiments
By injection of *Boophilus annulatus microplus* he recovered virus up to 10
days after their infecting meal but he again failed in transmission experi
ments In the single experiment attempted with chicken mites he fed these
on an infected monkey Seven days later they were ground up and injected
into another monkey which died of yellow fever

In a recent series of experiments in Brazil restricted to *A cajennense*
virus was recovered from the bodies of nymphs infected as larvae and
in one instance from the bodies of adults infected 2 months previously
as nymphs In no case was the virus of very high titer in the specimens
tested nor was it transmissible by bite but it was retained through one meta
morphosis and over a significant period of time

While the experiments discussed above do not indicate that ticks and mites
play any role in the maintenance of yellow fever in nature they do suggest
that aside from mosquitoes *Acarinae* are better able to retain the virus of
this disease than any other group of arthropods studied This coupled with
the knowledge that ticks can harbor other viruses and can transmit them
transovarially makes further studies on this group imperative

CONCLUSION

From the foregoing sections it is obvious that mosquitoes are of the ut
most importance in the transmission of yellow fever both in

type) and in the forest regions (jungle type) In addition it is undoub true that as far as man is concerned and perhaps all primates the mosq is the only arthropod that plays the role of reservoir vector In labora studies it has been found that several species of mosquitoes from at least genera (*Aedes Haemagogus Taeniorhynchus Eretmapodites Culex Trichoprosopon*) are capable not only of becoming infected with the virus yellow fever but of transmitting the virus by bite to susceptible vertebrat Furthermore field studies have shown that certain of these species have geographic distribution and ecology favorable to bringing them in contac with forest primates and with man at a time and place that would facili tate their transfer of virus from one vertebrate host to another Finally indi vidual members of some of these species which have been infected in nature have been captured and brought to the laboratory where they have trans mitted yellow fever to nonimmune monkeys by their bite Thus theoretical possibilities have been confirmed with actual observation

With regard to urban yellow fever in which the only vertebrate host is man the insect reservoir vector is predominantly *A aegypti* a fact so well proved as to need no further comment Whether other vectors can fulfill this function in South America is still open to question In certain instances it has been suspected that *A scapularis* and perhaps *A fluviatilis* because of their facultative domesticity can at times transfer yellow fever from man to man although no proof of this is available Similarly under unusual circumstances *Haemagogus* of several species might conceivably do the same However the possibility that these species could maintain an urban type of yellow fever epidemic is remote

In West Africa *A aegypti* is the mosquito commonly involved in the human to human transfer of virus On the other hand a severe epidemic of yellow fever occurred in 1940 in the Nuba Mountains of the Anglo-Egyptian Sudan which was undoubtedly aided and abetted by other mosquitoes in particular *A vittatus* a mosquito breeding in rock holes It was also thought that *A metallicus* and *A taylori* might be implicated In Eas Africa in the Bwamba area of Uganda *A simpsoni* undoubtedly takes the place of *A aegypti* in transmitting yellow fever from man to man This is acilitated by its custom of breeding in the plant axils of bananas and olocasia planted around the native huts

Turning now to jungle yellow fever there is a limited group of mosquitoes at is believed to be responsible for the transmission of virus from primate

to primate and from primate to man In South America these mosquitoes are of the genera *Haemagogus* (*spegazzinii spegazzinii falco capricornii equinus splendens*) and *Aedes* (*leucocelaenus*) They have in common a pattern of daytime feeding which brings them in contact with man and preference for the forest canopy (contact with monkeys) which is disturbed by such occupations as lumbering and the harvesting of palm hearts again favoring contact with man They may be found in the forest canopy at all seasons of the year thus permitting a continuous cycle of transmission should other conditions be favorable Naturally infected specimens have been repeatedly captured in both Brazil and Colombia

In the forested sections of Africa as represented by Bwamba County in Uganda somewhat different conditions exist which are presumably met by a slightly more complex cycle In this area two species of mosquito have been incriminated—*A africanus* and *A simpsoni* Of these *A africanus* is the arboreal species having a maximum contact with arboreal primates However unlike the South American *Haemagogus* its feeding time is sharply restricted to a period shortly after sunset a time that is particularly favorable for biting monkeys as they settle for the night Furthermore it shows a strong food preference for monkey blood over that of man and other baits Thus while potentially effective for transferring yellow fever from monkey to monkey it is not ideally adapted to bringing it from monkey to man To accomplish this latter *A simpsoni* is peculiarly well adapted Breeding in the peridomestic bananas and colocasia it has a close association with man But it frequents the margins of the forest surrounding the cultivated areas and has opportunity to feed on monkeys either in the forest edge or when they leave the forest to raid the plantations It thus can take blood meals from monkeys or man more or less at will That these two species transmit yellow fever in nature has been proved as in South America by the repeated isolation of the virus from wild caught specimens

Beyond these two *Aedes* we cannot go although through analogy we may suspect that *A luteocephalus* can aid or supplant *A africanus* as a vector Ecologically both in choice of environment and in feeding habits these two mosquitoes are similar Both transmit the virus by bite readily in the laboratory Furthermore as stated earlier both are closely related taxonomically to a degree where Haddow and Mahaffy (1949) considered them merely as extreme examples of a single species complex However as yet *A luteocephalus* has not been found infected in nature

But does this information offer a completely satisfactory explanation of the overall survival and dispersion of yellow fever in nature? What are the weak points in the chain of a continuous mosquito—vertebrate cycle of virus transfer?

In the first place the mosquito is considered the reservoir as well as vector of the virus since once it is infected it remains so and can transmit the agent again and again throughout its life span But what is its life span in nature? While laboratory caged mosquitoes free from predators and adverse climatic conditions may live for 3 to 4 months under optimal conditions all studies on the recapture of marked and liberated mosquitoes indicate a very short average life This would imply that the reservoir was effective only for a short time and that repeated transfer throughout the year was essential to prevent the dying out of the virus This repeated transfer necessitates an adequate supply of susceptible vertebrates to maintain the virus Does such an adequate supply exist particularly in areas where the immunity level among primates is known to be high?

Secondly does the available information on the dispersion of mosquitoes and vertebrates adequately cover the facts about the dispersal of yellow fever in nature? How did the virus of yellow fever get to the Nuba Mountains? Was it constantly present there in a mosquito—vertebrate cycle or was it introduced? If introduced by what mechanism did this occur? In most of the studies on dispersion of marked mosquitoes the majority of the recaptured insects have been taken within a distance of a mile from the point of liberation and for only a few days after liberation However Causey Kumm and Laemmert (1950) gave figures to show that individual mosquitoes may be recaptured as far as 11 5 km (approximately 7 5 mi) from the release point In dispersal studies it has been noted that the direction of the capture is associated with the direction of the wind and it has been assumed that dispersion is primarily a matter of the mosquito s being blown around What is of concern therefore is whether mosquitoes released from cages are more prone to be trapped by wind currents than the naturally occurring mosquito fauna in any area Secondly what percentage of the population is thus subject to dispersion? For the moment at least it would seem that available information although suggestive is inadequate to settle the question either in favor of or against the dispersion of the virus of yellow fever by means of infected mosquitoes

Turning now to studies on other insect borne virus diseases the work

done in the United States on the insect vectors of equine encephalomye
and St Louis encephalitis viruses will be found impressive The ear
evidence on these diseases pointed toward a mosquito vector as far as
and other mammals were concerned However as birds were found to be
ceptible to the virus bird parasites were investigated with the finding
bird mites of several species could be infected Furthermore viruses
isolated in nature from the mites in chicken coops as well as from rece
occupied nests of wild hedge birds Laboratory studies showed that the m
would transmit the viruses transovarially to the next generation (Sn
Blattner and Heys 1947) Thus there exists a potential reservoir of viru
markedly greater efficiency than any yet demonstrated for yellow fever
experimental point of interest has been the demonstration that the St L
virus could be passed through the egg to the third generation in the
Dermacentor variabilis (Blattner and Heys 1944) This finding is per
academic in so far as the epidemiology of St Louis encephalitis is concer
but does indicate the over all relationship of *Acarinae* to a virus disease

Returning now to yellow fever it is impressive that the virus can sur
for so long a period of time in ticks such as *Boophilus Argas Rhipicepha*
and *Amblyomma* and that N C Davis (1938c) was able to recover virus f
a group of unidentified chicken mites one week after their infectious m
However the fact that no transmission was obtained from these ticks by
has led investigators to discount the significance of the survival of the v
in them Furthermore the investigation of bird mites has been all but
nored because of the demonstrated fact that adult birds fail to circulate
low fever virus Perhaps investigators have been too hasty in passing up
field It is of interest that Lmbarcs (1943) was able to maintain the viru
yellow fever (neurotropic variant) by passage in day-old chicks but fa
with older birds In later studies it was found that even by the 2d
chicks were less susceptible to virus Similarly there is voluminous evide
(Bugher 1941) that day old mice are infinitely more susceptible to
parenteral injection of yellow fever virus than even mice 3 weeks of
Needless to say this leads to speculation as to the susceptibility of ne
hatched birds of those species which emerge from the egg at an earlier
bryologic stage of development than chicks Is it possible that such fle
lings could circulate yellow fever virus in sufficient amount to infect th
mites And could the next generation of fledglings be infected by the

exist it might offer an alternative explanation for the long time survival of yellow fever virus and its dispersion

Admittedly this is pure speculation and further amplification at this time is not justified However until the possibilities suggested have been further explored they cannot be ignored

6 THE MAMMALIAN HOST IN YELLOW FEVER

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analogous to the mild influenza like infections in human beings during an epidemic of yellow fever

On the basis of Thomas work Sir Patrick Manson (1909) raised the question of the importance of forest animals especially monkeys in yellow fever He suggested that if the virus could be carried by mosquitoes from man to the animals it could be carried in the reverse direction thus complicating greatly the problem of control

Several years later Balfour (1914) was struck by the prevalence of the belief among the Negroes of Trinidad that prior to an epidemic of yellow fever a considerable number of red howler monkeys would be found dead or dying in the forest The validity of this belief was confirmed by Urich entomologist to the Board of Agriculture in Trinidad who had seen monkeys lying dead at the time of the yellow fever outbreak the previous year but had not associated them with the epidemic Balfour mentioned that the Trinidad forests harbored *Stegomyia sexlineata* a mosquito closely related to the known yellow fever carrier He was sufficiently impressed by the possibility of wild monkeys acting as reservoirs of yellow fever virus to suggest to the British Colonial Office that the subject be studied among the monkeys of West Africa This indeed was made part of the program of the Yellow Fever Commission to the West Coast of Africa but was not accomplished due to the interruption of the investigations by the beginning of the First World War (Fowler 1931)

While these earlier studies and observations were highly suggestive conclusive experimentation awaited the identification and availability of the causative organism of the disease a susceptible experimental animal suitable for large scale laboratory use was also needed The isolation of the Asibi strain of virus in the rhesus monkey by members of the West Africa Yellow Fever Commission working in Nigeria and in the Gold Coast met both of these requirements The circumstances of this achievement which marked the beginning of the modern history of yellow fever have been related in a previous chapter

GENERAL CONSIDERATIONS OF THE ANIMAL ROLE

Fundamentally the purpose of all yellow fever investigation has been to elucidate the mechanisms by which the virus may infect man and thereby make possible effective prevention of the disease Yellow fever is of conse

quence to man only as it infects human beings. There is not the economic problem arising from losses of domestic animals which occurs in some other virus diseases such as Rift Valley fever. Underlying all the yellow fever studies therefore has been the need to determine the degree of risk to which individual human beings may be exposed. And it follows that the studies of the susceptibility of animals to the virus of yellow fever have been made from an epidemiologic point of view. The aim has been to establish whether and in what measure a given species of animal may be important in maintaining the virus in nature and in making it accessible to man.

It is immediately apparent that several basic conditions must coexist if a natural cycle of virus transmission is to be maintained. These are:

1. The animal in question must be susceptible to the virus. When a small quantity of the virus is introduced into the animal, multiplication of the virus must result; that is, a true infection must occur.
2. Virus in an infected animal must be transferable to other animals, either directly or by mediation of a vector such as an arthropod.
3. In the case of a mediating organism or vector, the circumstances must be such as to permit the transference of virus from the animal to the vector. This is equivalent to stating that host and vector must share the same habitat for at least a portion of the time.
4. The vector must be able to transfer the virus to other susceptible animals of the same or other species with sufficient frequency to permit continuity of the cycle.

These principles merit further consideration in order that the question of animal susceptibility may be properly evaluated.

ANIMAL SUSCEPTIBILITY

For the purposes of this discussion, an animal is considered susceptible to yellow fever virus when the introduction of virus into the animal is followed by an unequivocal multiplication of the agent. Pathogenesis is not necessarily implied. As a matter of fact, an animal with a high level of virus multiplication and no accompanying illness would probably be more successful as an intermediate host than one sick enough to be immobilized and incapacitated. The demonstration of virus multiplication is therefore essential. Unfortunately, we have as yet no quantitative method for estimating the number of virus particles in a sample of material and are forced to rely on measure

ments of the infectivity of the material for other animals In practi has been done chiefly by the intracerebral inoculation of the suspect m into white mice For more precise results groups of animals have been i lated with graduated dilutions of the infectious material and mortalit been used as the measure of infection The unit of virus is generally def as the amount contained in an inoculum that in the long run will kil per cent of the test mice This unit is known as an LD_{50}

The relation of the LD_{50} which is based on lethal infection to the actu number of virus particles is a highly variable one It depends upon th genetic character of the mice used (Sawyer and Lloyd 1931 Lynch an Hughes 1936) upon the age of the mice (Theiler 1930*b* Davis 1934*c* Bugher 1941 Whitman 1943) and upon the strain of virus (Theiler 1930*b*) It may be demonstrated that many strains of yellow fever virus produce nonlethal and immunizing infections in mice (Fox 1943) With some strains the mortality is so irregular that no endpoint can be shown

The biologic estimation of virus quantity is thus a highly variable procedure However reasonably consistent results may be obtained when comparisons are restricted to one strain of virus and a single stock of animals But since the results are comparative and not absolute it is unsound to apply the quantitative conclusions derived from one system to a different one

CHARACTER OF THE ANIMAL INFECTION

In view of the fact that the natural maintenance of yellow fever virus appears to depend overwhelmingly on an arthropod vector notably certain mosquitoes the presence of free virus in tissues and body fluids easily acces sible to the vector becomes of paramount importance Consequently mo studies of animal susceptibility have emphasized the circulation of vir n the blood stream rather than factors such as specific tissue affinities of th irus and pathologic changes resulting therefrom

Because of the multiplicity of factors controlling the final importance of a sceptible animal in natural cycles it does not follow that an animal that ibits virus multiplication and is therefore susceptible is necessarily a nificant component of the natural history of the virus Evidence is also led as to the frequency with which the particular animals are actually ted in nature There are two possible ways of getting such evidence testing captured specimens for existing infection as manifested by the

presence of virus and (*b*) by examining captured specimens for signs of past infection by means of immunologic tests

The first procedure is of little value without some method of preliminary screening to limit the coverage Mouse inoculations with sera of wild monkeys in East Africa and with sera of wild monkeys and marsupials in Colombia gave no positive results in spite of the known presence of yellow fever Even *with screening there has been only one instance of successful* virus isolation from a captured animal Laemmert de Castro Ferreira and Taylor (1946) in Brazil were able to demonstrate yellow fever virus in a marmoset

More fruitful results have been secured through the use of the neutralization test On the basis of the experience with the mouse protection or neutralization test in developing the epidemiology of human yellow fever it has been generally accepted that similar methods would be of great value in tracing the epidemiologic lines among the animals

Consequently yellow fever studies of wild animals have been definitely oriented along lines that would afford information of greatest epidemiologic value even though knowledge of the character of the infection and the course of the pathologic process in the animal itself might be left incomplete with this approach With reference to the development of antibodies in the various species great attention has been given to such questions as specificity antibody persistence and duration of immunity But conclusions that are valid for one species obviously cannot be extended to other species even of the same genus without careful experimental checking

RELATION OF ANIMAL TO VECTORS

An animal that is circulating virus in quantities sufficient to infect at least some of the vector population may still be of no importance in the natural cycle if its habits are such that the vector does not have access to it The ecologic relations between the animal host and the vector are critical determinants in the epidemiologic situation and must be fully understood for a clear picture of the epidemiology Host and vector must share the same habitat under conditions that permit the vector to acquire the virus This is a four dimensional relationship both space and time being involved and is especially well illustrated by the man monkey and mosquito (*Aedes africanus*) complex in the forest of western Uganda (Smithburn Haddow and Lumsden 1949) The proved vector *A africanus* is a crepuscular biter

with a predilection for the forest canopy At the time of peak biting a the human beings have left the forest itself for the cleared villages and ments, while the monkeys retire into the canopy in their sleeping trees sequently, the monkey cycle with this mosquito is a dominant one, whil man–mosquito cycle occurs rarely at best Therefore the area has a high monkey infection rate associated with a very low human infection r

CHARACTERISTICS OF THE VECTOR

For successive virus cycles to be maintained, the vector must becom infected and transmit the virus often enough to ensure that infected indi viduals of the vector class exist constantly The minimal level of transmis sion for virus maintenance has been studied only casually. There are no quantitative data save for the man–aegypti cycle, and even these figures are incomplete

The minimum vector infection rate required to maintain endemicity ap pears to vary, being greatly influenced by such factors as longevity of the vector, its flight range and population density, and by the character of the animal host population itself, particularly with reference to the proportion of immune animals Natural conditions are clearly very different from ex perimental ones An event of low probability in the laboratory may be appreciable in frequency under natural circumstances because of the large population within which the probability system operates Infection rates that fail to maintain virus cycles under laboratory conditions, where single animals and small samples of mosquitoes are used, may be successful under natural conditions, where the animal population may be measured in thou sands and the mosquito bites in millions These ultimate epidemiologic considerations have, of course, influenced the approach to the problem of animal susceptibility and to the problem of animals as hosts to the yellow ever virus

FURTHER GENERAL CONSIDERATIONS

There are still other factors that may influence the behavior of an ani l with respect to yellow fever virus Mammalian taxonomy is relatively de, and it is generally recognized that a taxonomic species may vary iderably from one geographic region to another At times there may gnificant difference of opinion among experts as to whether two indi

viduals should be assigned to the same or to different species. It may be expected therefore that within what appears to be a perfectly homogeneous species there may be marked differences in susceptibility to the same virus strain. The importance of genetic composition has been demonstrated by selection studies within an established inbred line of albino mice; genetic composition may be presumed to exert even greater influence in a wild population.

Similarly, it may be anticipated that within a single geographic region the same species will vary over a long period of time in its susceptibility to a given strain of virus. Repeated catastrophic epidemics may favor the numerical predominance of relatively resistant individuals. Hence the population as a whole may appear to be less susceptible than other populations of the same species, although its resistance is not based upon active immunity. There are possible examples of this in human epidemiology; people native to communities with long histories of yellow fever have been observed to have the disease in milder form than newcomers from regions free of the disease (Carter, 1931). Similar evidence among animal populations is difficult to assess. A possible example is the apparent resistance of the small Nigerian bush baby, *Galago demidovii demidovii*, in comparison with the other *Galaginae*. The only galago in intimate contact with endemic yellow fever in Africa, it exhibits a very low level of susceptibility. It is the only primate so far studied that has failed to show clear susceptibility to the local virus.

The rate of reproduction may also be an important factor. It determines not only the population density, but also the frequency with which susceptible individuals are added. Large and frequent litters are usually associated with short life span, so that among animals such as the South American marsupials, which twice a year bear litters of eight to ten, the population is highly fluid in comparison with the monkeys. Again, the turnover in the monkey population is much more rapid than among the human population of the same area. It has been noted that with aegypti-transmitted epidemics in African villages the outbreak spontaneously ceases at an immunity level of approximately 50 per cent. However, in the Bwamba forest of Uganda an extensive epidemic among the monkeys began and continued (Smithburn, Haddow, and Lumsden, 1949) in a region that had previously shown an immunity proportion of nearly 60 per cent (Haddow, Smithburn, et al., 1947). It is probable that such occurrences reflect population turnover more than they reflect vector efficiency.

Apart from the major ecologic relationships apparently incon habits of the animal may have a significant bearing upon the freque fection This was well illustrated by a simple experiment of Mattin placed a *Perodicticus potto potto* and a *G demidovii demidovii* lar cages into each of which he introduced 10 africanus mosquitoe the same catch The indolent potto was bitten successfully within a time by most of the mosquitoes while the active and aggressive galago s caught and ate all of the mosquitoes in his cage Animals that are ex ingly active during the peak biting period of the mosquito vectors may expected to receive virus by bite with less frequency than more lethar species

Although not fully appreciated at first one of the determining factors i the apparent susceptibility of any animal is the strain of virus presented t it Studies by Anderson by Laemmert (1911) and by Waddell and Taylor (1916) have demonstrated that marked differences in the responses of certain animals may be due to strain variations and that species moderately sus ceptible to one strain may be quite refractory to another

TAXONOMY

Among the recurring difficulties in a discussion such as this is the matter of nomenclature where the virologist is necessarily at the mercy of the taxonomist A vast amount of labor has been expended in the field of taxonomy but considerable confusion still exists with many of the orders and will probably continue for a long time to come Despite the policy of accepting the classification of some reasonably authoritative publication and then adhering to it for as long as seems justifiable there remains a good deal of variation in the naming of the same animal Not only do the experts differ in their preferences but a single taxonomist may revise his opinion with time Since authors may consult different taxonomists or the same taxonomist at different times considerable variation in the designation certain animals may ensue A good example is the present position the genus *Cebus* in South America The number of valid species varies m two to over twenty in the eyes of different authorities. The taxonomy he African monkeys is still less settled with disagreement as to the num of genera that should be allowed

ed with such a situation the medical virologist must make a number itrary decisions and adhere to them The terminology of this chapte

will therefore be that which has been most commonly used and which will in the author's opinion, be intelligible to the greatest number of readers even though, in the process, rigorous principles of taxonomy and even logic are violated Since jungle yellow fever appears to be limited to Africa and South America with the contiguous Central America the consideration of the susceptible animals will be primarily upon a continental basis



Fig 31 Localities in Brazil where yellow fever immune primates have been captured.

THE SOUTH AMERICAN VERTEBRATES

PRIMATES

The monkeys of the New World belong to the suborder *Anthropoidea* Except for the family *Callitrichidae* they are distinguished from those of the Old World by two characteristics the number of teeth (36) and the posses

sion of a prehensile tail South American monkeys divide into two fa
(one of which is very large and is further subdivided into four subfam
and fall into the following general outline (Elliot 1913)

Family *Callitrichidae*

Genera 1 *Seniocebus* (bald headed tamarins)
2 *Leontocebus* (black tamarins and tamarins called al
lion marmosets)
3 *Oedipomidas* (marmosets)
4 *Callithrix* (true marmosets)
5 *Callicebus* (widow monkeys)

Family *Cebidae*

Subfamilies

A *Alouattinae*
Genus *Alouatta* (11 species) (howling monkeys)

B *Pitheciinae*
Genera 1 *Pithecia* (8 species)
2 *Cacajao* (3 species)
3 *Saimiri* (7 species) (squirrel monkeys)

C *Aotinae*
Genus *Aotus* (11 species) (night monkeys)

D *Cebinae*
Genera 1 *Ateles* (11 species) (spider monkeys)
2 *Brachytelus* (1 species) (woolly spider monkey)
3 *Lagothrix* (6 species) (woolly monkeys)
4 *Cebus* (21 species) (capuchins)

Family *Callitrichidae*

The tamarins and marmosets make up this family which is widely dis
outed from Panama through many parts of northern South America They
small animals with rounded heads conspicuous ears and small faces
relatively large eyes and mouths and are further characterized by 32
in the full dentition They are quick restless and in general timid
ugh they may be easily tamed and become attached to human beings
do not survive well in captivity A number of the *Callitrichidae* have
ested for their response to yellow fever virus

Leontocebus ursulus (Humboldt)

Black tamarin. Called also *Cercopithecus ursulus* (Humboldt) by Elliot. Lower Amazon region; type locality, Para. Adult length including tail approximately 40 cm. Pelage dark, predominantly black with brownish red tinge over the back in some specimens.

N. C. Davis (1930*b*) studied five specimens of this species, using the Asibi strain of yellow fever virus. The first was inoculated subcutaneously with a liver emulsion from a rhesus monkey infected with Asibi virus. Two days later the animal had a high fever, and blood transferred to a normal rhesus monkey resulted in death of the latter from typical yellow fever. Aegypti mosquitoes fed on the same day later transmitted fatal yellow fever to a rhesus monkey. The leontocebus died on the 9th postinoculation day, and on the basis of the gross and microscopic pathologic findings, it was concluded that the animal had died of yellow fever.

A second *L. ursulus* was infected by the bite of aegypti mosquitoes previously fed on a rhesus monkey with yellow fever. This animal had fever on the 3d day and died on the 13th day. Autopsy revealed typical yellow fever changes. The virus was passed by blood transfer from this second *L. ursulus* in series through the remaining three, all of which developed fever and severe illness and died. Aegypti mosquitoes fed on the 5th animal transmitted a fatal infection to a rhesus monkey.

Since none of the animals survived, it was impossible to draw any conclusions concerning their ability to produce antibodies. It was evident from these experiments that *L. ursulus* had the same order of susceptibility to the Asibi strain of virus as the rhesus monkey.

Leontocebus rosalia (L.)

Tamarin. Widely distributed in Brazil, including State of Rio de Janeiro forests of the upper Amazon and forests of southeastern Brazil. Adult length about 60 cm over all, of which a little more than half is tail length. Head, body, and legs golden yellow, becoming lighter caudally. Bushy terminus of the tail darker than the cephalad portion. Face, hands, and feet dark brown with purplish tinge. There are long tufts of brownish black hair inside ears.

Laemmert (1944) conducted susceptibility studies of *L. rosalia* using four strains of yellow fever virus. He found a striking difference in mortality between the two African strains and the two strains from South America. None of 20 animals inoculated with the French and Asibi strains died, while all of

the 11 animals that received the Brazilian strains succumbed. With
can viruses, two animals receiving the smallest doses of the Frenc
failed to produce neutralizing antibodies, but all of the others dev
positive protection tests.

In another experiment with *L. rosalia* and the same four virus st
all of the animals had virus circulating in the bloodstream from the 2d t
6th days, and in most circulating virus persisted through the 7th day
survivors developed neutralizing antibodies.

Oedipomidas oedipus (Fig. 35)

Colombian marmoset. Does not occur east of the Andes but is to be found in the lower Magdalena valley, the Sinu River valley, and the coastal region near Cartagena. Small, similar to other marmosets. Tail longer than body. Crest of long white hair on head extending to nape of neck. Arms, outer portions of legs, hands, and feet white. Upper parts variably grayish brown; considerable color gradation in tail.

Bates and Roca Garcia (1946*a*) used four oedipomidas for interposition in yellow fever virus (Rodas strain) cycles with haemagogus mosquitoes and aotus or saimiri monkeys. All four animals circulated virus in very large amounts, the titer reached 10^{7} in one animal and 10^{5} or more in the other three. Three of the four died of the infection and showed a diffuse necrosis of the liver at autopsy. The serologic reaction of the surviving animal was not reported.

The level of susceptibility of this marmoset is clearly very high and similar to that of the related genera. It may be presumed that the closely related *Oedipomidas geoffroyi* of Panama and the Atrato River Valley has a similar response to the virus, although no susceptibility tests have been reported. Of 24 *O. geoffroyi* sera collected along the Napipi River by Bugher and Boshell Manrique, two gave positive protection tests.

Callithrix jacchus (L.)

Common marmoset. Originally described from the Island of Marajó, Brazil. Classification in dispute; some closely related species may well be color and eographic variants of the same stock. In typical specimen face black with hite spot; head, nape, and neck brownish black. Ears naked with white tuft airs. Lower back barred ochraceous, black, and grayish white; tail banded ack and white. Total length about 51 cm., of which nearly three fifths is l



Fig 35 *Oedipomidas oedipus* (Colombian marmoset)



Fig 36 *Callicebus ornatus* (widow monkey)



Fig 37 (Above) *Alouatta caraya* (Brazilian howler monkey) Male and female



Fig 38 (Right) *Saimiri sciureus caquetensis* (squirrel monkey)

This species was very carefully studied by Laemmert (1911) in wh
some points of view were the first adequate investigations of the r
of the genus *Callithrix* to the virus of yellow fever A total of 172 anim
used in experiments that embraced the susceptibility to various str
yellow fever virus the virus levels attained in infected animals and an
response All animals were subjected to a pre experimental protection
using the young mouse test of Whitman (1913) Only those giving a neg
test indicating the absence of antibodies were used in the experiments

Laemmert used a total of eight African and South American virus stra
which represented the spectrum of the viscerotropic viruses as they have be
actually encountered in outbreaks in the two continents These were th
Asibi (British West Africa) the French (Senegal) the JF (Brazil) th
MAJ (Brazil) the J7 (Brazil) the OC (Brazil) the Martinez (Colombia)
and the AC Bol (Bolivia) strains The method of infection was the sub
cutaneous inoculation of virus preparations of rhesus monkey origin The
virus materials were assayed by mouse titration so that the doses could be
expressed in terms of mouse LD$_{50}$ units Some of the animals were bled
daily for quantitative estimations of the circulating virus

There were no significant differences in the infectiousness of the various
strains for the marmosets 133 out of 137 animals either died of yellow
fever or responded by developing antibodies However Laemmert s studies
brought out very clearly the significance of strain differences in other re
spects There were for example marked differences in mortality rates with
the various strains as shown in Table 9 (Laemmert 1911)

The low mortalities with the African strains are outstanding in view of
the fact that both produce a very high mortality (approaching 100 per cent)
in the rhesus monkey In contrast were the high mortalities caused by all
the South American strains except the Martinez strain Virulence was not
correlated either with the number of rhesus passages or the previous his
tory in mice

Strain differences were also strongly reflected in circulating virus levels
our strains of virus—Asibi French OC and J7—selected as typical Afri
n and South American varieties were studied quantitatively by Laemmert
he highest and most prolonged circulating virus titers followed a small
e of the Asibi strain circulating virus persisting for 8 days In general
smaller doses gave the longer circulation times especially with the

African strains. With the South American strains death supervened so early that the full picture with respect to circulating virus levels did not have a chance to develop.

TABLE 9

MORTALITY OF CALLITHRIX JACCHUS INOCULATED WITH DIFFERENT YELLOW FEVER VIRUS STRAINS

Number of marmosets inoculated	Virus			Result					
	Strain	*Number of passages in rhesus*	*Range of LD_{50} of virus inoculated*	*Died of yellow fever*		*Survived*		*Non-specific death*	*Yellow fever [illegible]*
				Number	*A.S.T.**	*Number*	*Number immune*		
16	A b	39 to 43	1 × 10^6 to 1.1 × 10^6	0	8.0	13	12	3	0 2 0
16	As'b	17	6 × 10^3 to 6 × 10^5	3	5.6	12	11	1	5 14 21
22	F en h	±17	2 × 10^0 to 1.5 × 10^6	5	6.0	14	13	3	5 18 8[illegible])
22	J Z	3 to 5	1 × 10^0 to 6.6 × 10^4	20	7.5	2	2	0	20 22 9
18	O C.	1 to 5	3 × 10^1 to 2.7 × 10^4	15	8.1	3	2	0	15 1 [illegible]
15	J F	11	2 × 10^2 to 1 × 10^5	7	9.0	5	5		12 58
12	M A J	21	1.2 × 10^3 to 1.2 × 10^5	9	7.0		3	0	9 2 7
14	A C Bol	1	4.8 × 10 to 4.8 × 10	11	9.3	1	1	2	11 2 92[illegible]
13	Martínez	3	3.5 × 10 to 5 × 10^3	3	6.6	5	5		3 8 38

*A.S.T. = average survival time in days

As a further experiment, aegypti mosquitoes were allowed to feed on infected marmosets; the insects later transmitted the virus to other marmosets without difficulty. The mortality experience was the same as with injected virus. In all Laemmert's experiments, as well as in the other investigations that have been reported, there is no evidence that mosquito passage in itself modifies the virus. As far as the animal is concerned, there is no distinction between virus introduced by mosquito bite and a corresponding amount injected by syringe.

Callithrix albicollis (Spix)

Marmoset. Region of Bahia, Brazil. Total length about 50 cm., of which the tail comprises 30 cm. Ears tufted. Neck hairs long, forming a mantle. General coloration reddish brown, with light gray ear tufts, neck, and sides and back of the head. The closely related *Callithrix leucocephala* (Geoffroy) and *Callithrix humeralifer* (Geoffroy) are considered by some to be color variants of *C. albicollis* rather than separate species. The Brazilian marmosets used by Stokes, Bauer, and Hudson (1928*b*) in West Africa in the attempt to isolate yellow fever virus probably were *C. albicollis*.

the possibility that they might enter into a forest cycle of the virus w various wild species of mosquitoes that had been shown to be efficie vectors

Callithrix aurita (E Geoffroy)

White-eared marmoset Originally described from the State of São Paulo found also in the State of Rio de Janeiro apparently inhabits the littoral of the two regions Somewhat larger than *C jacchus* Ears tufted with long white hairs Face and forehead yellowish white the vertex and nape of the neck ochraceous, rest of head, neck back between shoulders and lower parts of back black remainder of back and portions of extremities tawny and black mixed, tail banded or ringed transversely alternating black and gray

These animals were studied by Waddell and Taylor (1946) for their abil ity to take part in *A aegypti* and *Haemagogus equinus* transmission cycles The O C strain of virus which had been shown by Laemmert (1944) to be especially virulent for marmosets was used and successful transmission maintained through nine cycles with *A aegypti* In no instance did an ex posed marmoset fail to become infected, all showed virus in the blood when first tested on the 2d or 3d day Fifteen of the 17 exposed animals died between the 4th and 8th days, and virus continued to circulate in their blood until death The two survivors gave a positive protection test with sera taken on the 21st day following exposure There was no diminution in the severity of the disease as the passage progressed, the serum of the last animal in the series had a virus titer of more than 10 ⁷

From the 7th aegypti–marmoset cycle a series of passages with *H equinus* was initiated, carried through three cycles and then terminated One cycle was made with *Haemagogus spegazzinii*, also successfully

These experiments were designed to show the facility with which trans mission cycles could be maintained with this marmoset and both urban and forest vectors However, they also demonstrated the high level of suscepti bility of the species, represented by the marked mortality The one virus titration figure indicated that the virus level was very high and entirely comparable to that of the rhesus monkey with Asibi virus It may be pre sumed that a great majority of the mosquitoes that fed on these animals were capable of transmitting yellow fever after the appropriate incubation time

Again the low proportion of survivors leaves us with little if

concerning the immunologic behavior of the species. However, none of the pre-experimental sera from these animals protected mice against yellow fever, whereas the sera of the two survivors definitely did give protection. Virus has not been found in the region from which these specimens of *C. aurita* came.

Callithrix penicillata (F. Geoffroy)

Marmoset. Has been described in Brazil from the States of Goiás, Minas Gerais, and Espírito Santo, and along the Paraná River. Also very numerous near Ilhéus in the southeastern region of the State of Bahia. Resembles *C. jacchus* in size. Face scantily covered with white hairs; forehead spot white. Head and back brown to black; upper parts and outer limbs gray; lower back and rump banded with black. Tail ringed black and white. Under parts black on throat and chest, gray on abdomen.

Studies by Waddell and Taylor (1946) followed the pattern used with *C. aurita*. Animals shown to be nonimmune by two previous negative protection tests were bitten by *aegypti* mosquitoes infected with the Almada strain of yellow fever virus. Virus quantitation was made by inoculating decimal dilutions of serum into groups of six Swiss mice. In one instance a circulating virus titer as high as 10^{-7} was found. Only three of seven animals studied failed to show a titer of 10 [illegible] or higher. The lowest maximum titer was $10^{-2.5}$. No difficulty was encountered in maintaining the series for five cycles, after which it was discontinued.

Of the eight animals exposed to infected mosquitoes, all became infected and six died. Although only one of the animals showed liver lesions that could be interpreted as being due to yellow fever, all six were considered to have died as a result of the virus infection. The two survivors gave positive postexperimental protection tests.

The importance of the marmosets in the epidemiology of jungle yellow fever in a particular region was shown by the very extended studies of Laemmert, de Castro Ferreira, and Taylor (1946) of an outbreak in the Ilhéus region of Brazil. Yellow fever virus was isolated from *C. penicillata* on four separate occasions over a period of 9 weeks from June to August 1944. The maximum distance between the areas of capture of the animals was 3 kilometers.

Callithrix leucocephala (E. Geoffroy)

White-fronted marmoset. Eastern coast of Brazil. States of Minas Gerais and Espirito Santo in eastern Brazil. Total adult length varies from about 40 to 50 cm., *of which the tail is approximately one half. Face very light; head* from ears forward, throat, and chest white; back of head and neck black; ears black with long black tufts. Upper parts of body ochraceous; hairs have black bar just below the yellowish-white tips, producing effect of a mixture of three colors. Tail ringed with gray and black bands.

Ten of these marmosets were tested with the Asibi, J.Z., O.C., and A.C. strains of virus. All but one died of yellow fever; the survivor received the Asibi strain and showed neutralizing antibodies.

Callicebus moloch (Hoffmannsegg)

Widow monkey. Brazil and adjoining portions of Venezuela, Ecuador, Colombia, and Peru. Small, sad-faced, with long, fine hair and somewhat bushy appearance. Exceedingly delicate; generally does not survive captivity well. Head and face small; ears large; tail long and bushy. Color brownish gray with orange-red cheeks, thorax, and belly.

Two animals thought to be of this species were inoculated by Davis (1931*b*) with Asibi virus. One was tested for circulating virus on the 4th day by subinoculation of blood into a rhesus monkey, which died of typical yellow fever. Also, a batch of aegypti mosquitoes fed on this callicebus later transmitted yellow fever virus by bite to a rhesus monkey. The callicebus showed a *moderate fever but no illness attributable to the infection, death* being due to pericarditis. The second callicebus showed no signs of illness but died a month later of undetermined cause. A blood specimen of the animal taken 17 days after inoculation protected a rhesus monkey against challenge infection. Davis concluded that *C. moloch* was susceptible and could take part in a transmission cycle, although it did not seem to be especially responsive to yellow fever virus.

Callicebus ornatus (Gray) (Fig. 36)

Similar to *C. moloch*, except that fur on head is coppery red behind white brow band.

In the general animal survey made of the Villavicencio area of Colombia in 1936–1937 by Kerr and Boshell Manrique, 11 specimens of this species were

collected Eight of these gave clearly positive protection tests interpreted as indicative of prior infection with yellow fever virus It was thought that the animals had probably been involved in the previous yellow fever outbreak near Restrepo

Bates and Roca García (1946*a*) obtained 25 *C ornatus* in 1944 in the same general region where the virus was known to have been present in 1943 Only three of the animals gave evidences of antibodies leading Bates and Roca García to conclude that *C ornatus* had not been extensively in volved in the 1943 outbreak

Infected haemagogus mosquitoes were allowed to bite four callicebus that did not demonstrate antibodies Two showed circulating virus in mod erate amount and both died early However one of the two survivors gave a strongly positive postexperimental protection test Two additional non immune callicebus were inoculated subcutaneously with the Perez strain of virus Both showed circulating virus in relatively small amount the high est titer attained on any day being 10^{2} Neither of the animals survived for a postexperimental protection test

From the small amount of work that has been done with this species it seems reasonable to assume that the serologic behavior is similar to that of the other primates of the region and that the positive protection tests are a reliable indication of infection with the virus

General considerations of the *Callitrichidae*

The studies of Taylor and his colleagues (1946) demonstrated that yel low fever may be widely transmitted among a marmoset population by haemagogus mosquitoes recognized as the forest vector of chief importance in South America (Shannon Whitman and Franca 1938 Bugher Boshell Manrique et al 1944) In large areas of Brazil marmosets are very abun dant and exhibit a rapid rate of reproduction Their number and close proximity to towns made them suspect almost from the beginning of the studies of yellow fever in wild animals (Davis 1930*b*) Given a suitable vec tor it would seem that the introduction of virus into an area with a large marmoset population should be followed by an explosive epidemic accom panied by a high mortality and resulting in near extinction of the various species In other areas of South America particularly the Andean zone marmosets are scarce or nonexistent although in these regions yellow fever may be endemic It may be said that the yellow fever pattern for marmosets is analogous to that of the local human populations

Pathologically the disease caused in the various species of marmosets and tamarins by South American strains of yellow fever virus is exceeding severe In most respects it resembles the illness produced by the Asibi strain in the rhesus monkey The outstanding lesions are in the liver where there is widespread coagulative necrosis of the liver cells with the formation of compact eosinophilic bodies from the necrotic material (Councilman bodies) Clearly defined zonal predilection of the necrosis is rare usually all zones of the lobule are diffusely involved at the same time Fatty changes are prominent the fat being distributed in small droplets in all zones in both necrotic and surviving cells Icterus is variable in degree but is usually present Marked degenerative lesions are also visible in other organs especially the kidneys where simple necrosis of the tubular epithelium may appear as the end stage of the very diffuse and severe cloudy swelling Hyaline and granular casts often icteric are encountered Splenic hyperplasia may occur accompanied by an increase in large mononuclear cells

Although large numbers of *Callitrichidae* have been used in experiments very little has been recorded of the actual quantitative results obtained in protection tests When animals recovering from known virus infection have been tested and found to have neutralizing antibodies or have been designated immune the presumption is that the results of the protection tests have been given a positive interpretation On the basis of published statements it is probable that the general behavior of the *Callitrichidae* in protection tests is similar to that of the rhesus monkey The reactions would appear to be entirely specific if ordinary care is taken in collecting the sera nonspecific reactions appear to be relatively rare No information is available on the duration of the humoral antibodies reported tests extending over short time intervals only However the epidemiologic interpretation of field tests has rested upon the assumption that antibodies persist for life since such seems to be the case with most of the other South American primates It is unfortunate that there are no direct data because this point is of considerable consequence in the evaluation of the Ilheus study (Taylor onseca da Cunha et al 1946) where a low proportion of animals with positive protection tests was associated with active epidemicity of a virus am producing high mortality

Family *Cebidae*

Subfamily *Alouattinae*

Howling monkeys Subfamily consists of a single genus *Alouatta* but there exists considerable divergence of opinion concerning the separation of the different species Distributed widely from about the level of Veracruz Mexico in the north through Central America and the Isthmus of Panama down to the entire northern portion of South America found in Brazil the Guianas Venezuela Colombia Ecuador Peru Bolivia Paraguay and Argentina Geographic ranges of the various species overlap a good deal Large heavy animals distinctly brachymorphic Head large skull especially the mandible heavy Laryngeal and hyoid structures large and cavernous permitting the deep resonance characteristic of the call of the *Alouattinae* Tail long prehensile and quite powerful serving at times to suspend the entire weight of the animal from the limb of a tree Thumb well developed and opposable Face naked with heavy beard beneath the chin especially in old males Coloration may vary sometimes markedly from black to yellowish red both from species to species and within a given species or even within a single band of animals hence coloration is a difficult and unreliable basis for species differentiation

Alouatta seniculus

Howler monkey Described originally from Cartagena Colombia widely distributed in Colombia and neighboring countries although total population always rather sparse and individual bands usually small and limited in range Largest of the New World monkeys Exhibits a great diversity in coloration coat varies from light yellow to deep purplish red back always lighter than head

Very few *A seniculus* have been studied for their susceptibility to yellow fever despite the early attention drawn to them by stories of their deaths during yellow fever epidemics (Balfour 1914) Davis (1931*b*) infected one monkey by means of mosquitoes carrying the S R strain of yellow fever virus Additional aegypti mosquitoes feeding on the howler became infected indicating the presence of circulating virus in quantity sufficient to infect at least some of the mosquitoes The animal appeared somewhat ill and had a fever but did not die Serum taken on the 17th postinfection day gave complete protection when tested in a rhesus monkey

Whitman further extended the observations by inoculating four mon

keys with Asibi virus. Two died of yellow fever and showed circulating virus in moderately high titer (10). A third monkey had circulating virus and fever but survived and subsequently gave a positive protection test. The fourth monkey showed no circulating virus or evidence of illness; the pre-experimental serum of this monkey was found to be strongly protective, indicating an immunity resulting from an earlier infection.

Alouatta palliata (Gray)

Black howler monkey. Components of *A. palliata* occur with moderate variability and have been described under various subspecies from the State of Veracruz, Mexico, through Central America and Panama to the Chocó in Colombia. Closely related to *Alouatta aequatorialis* (Festa) described from Ecuador and to the brown howler, *Alouatta ursina* (Humboldt) of Venezuela.

No direct experimentation with *A. palliata* has been reported, but the work of Courtney (1950) indicated that the species very probably plays a role in jungle yellow fever in Panama. Courtney has set forth the results of the studies of Clark, who found that a large percentage of the black howlers from the neighborhood of the Panama Canal had positive protection tests. While critical experimentation has not been done with any of the howler monkeys, it is reasonable to presume that their immunologic reactions may be interpreted on the same basis as those of other primates, and therefore that the positive protection tests are valid evidence that these monkeys have participated in the forest cycle of yellow fever.

That the black howlers of the Chocó have been involved in the forest cycle was shown during the Second World War by Bugher and Boshell Manrique, who made a survey of the coastal region of the Chocó. Of about 0 monkeys (mostly black howlers) taken, approximately 20 per cent gave ositive protection tests. It would appear, therefore, that the jungle yellow ver of Panama is a northerly extension of that of the Magdalena and Atrato ver Valleys in Colombia.

Recently Laemmert and Kumm (1950) have studied the susceptibility of n *Alouatta caraya* (Fig. 97) and three *Alouatta guariba clamitans* to the strain of yellow fever virus using *A. aegypti*, *Aedes scapularis*, and *es leucocelaenus* mosquitoes in cyclic transmission experiments. All 10 keys became infected and succumbed rapidly. Before death all the an had circulated virus in high titer, as determined by inoculations of dilutions of blood serum into groups of white mice.

Although *Alouattinae* as a whole have had very little study chiefly because of the difficulty of maintaining them in the laboratory it seems clear that they are very susceptible to the virus of yellow fever developing a severe and often fatal disease They circulate virus in abundance and infect mosquito vectors readily In those that survive the infection the immune response seems to be effective Judging by the results of protection tests of both captive and wild specimens the immunologic behavior of the *Alouattinae* is similar to that of man and to the great majority of other primates

Subfamily *Pithecinae*

Genus *Saimiri*

Squirrel monkeys also called *titi* in Colombia although the name applies to marmosets elsewhere Extensive range from Costa Rica in the north all through the upper watersheds of the Amazon and Orinoco through the Guianas to Bolivia in the south Found in moderately large bands of 20 to 30 near Villavicencio Colombia saimiri are commonly mixed with *Cebus fatuellus* Small attractive shy exceedingly active strictly arboreal and possessed of considerable curiosity Coloration generally light without much variation Consume a varied diet but particularly fond of insects and grubs

Saimiri sciureus (L) (Fig 38)

Most widely distributed and best known of the saimiris Originally described from Venezuela found in the Guianas Venezuela eastern Colombia and the upper Amazon Valley in Brazil Total adult length 70 cm of which slightly more than half is tail Face light colored covered by white hairs lips nearly black Head neck and back gray with brown or yellowish red mixture inner portions of arms and legs yellowish white belly usually still lighter nearly white Tail gray for most of its length with black tip

Aragao (1929*a*) reported that the Macaco do Amazonas (*Chrysthrix sciureus* here synonymous with *S sciureus*) was more susceptible to the virus of yellow fever than *Pseudocebus azarae* (here the same as *Cebus azarae*) In saimiri monkeys fever illness and frequently death resulted from the infection and the animals showed the classic liver lesions of yellow fever Davis (1930*c*) who also conducted experiments with the species obtained essentially the same results Davis employed both the Asibi and S R strains of virus and 10 of 15 animals inoculated died of the disease The temperature responses of the infected animals were inconstant and of little value as an indication of the severity of the disease The lesions

found in the livers of the fatally infected animals were comparable to those found in the rhesus monkey and were distinguished by severe necrosis most marked in the midzone of the liver lobule

Using 15 additional animals Davis (1930*c*) had no difficulty in securing transmission by the bite of aegypti mosquitoes The disease produced did not differ in any respect from the disease caused by direct inoculation of virus By means of the rhesus monkey protection test antibody was demonstrated in the blood of the animals that recovered and it was concluded that antibody formation was consistent and abundant

Lloyd and Penna (1933*b*) studied the behavior of *S sciureus* following the intracerebral inoculation of neurotropic yellow fever virus making animal-to-animal passages by filtered brain suspensions All the experimental animals developed fatal encephalitis From the pathologic standpoint the most significant lesions occurred in the central nervous system where there was extensive necrosis of nerve cells and neuroglia with marked perivascular round cell infiltrations The livers of these animals showed only moderate degenerative fatty change

At Villavicencio Colombia Bugher tested three saimiri monkeys with a local strain of virus Two animals showed moderate amounts of circulating virus (10^{-3}) and developed positive protection tests although they did not appear appreciably ill The third monkey did not react to the virus and its pre-experimental serum yielded positive results with the protection test Special attention was paid to the protection test behavior in order to obtain information for interpreting the results from the field surveys It was concluded that the *S sciureus* from the Villavicencio area behaved essentially the same as the sample studied by Davis From Davis results and the additional information obtained from the Villavicencio studies the protection test behavior of *S sciureus* was judged to be very similar to that of the rhesus monkey in that with both animals nonspecific reactions in well conducted tests are very infrequent and antibody formation is clear-cut

S sciureus were used in considerable numbers by Bates (1944*a*) to replace the expensive rhesus monkey in transmission experiments with mosquitoes Utilization of saimiri also made it possible to conduct the experiments entirely with locally obtainable biologic materials a very desirable end in itself Of 14 animals allowed to follow an unmodified course of infection with yellow fever five died a somewhat lower mortality than that observed by Davis

Subfamily *Aotinae*

Night monkeys, also called owl monkeys, douroucoulis, and sobrehumos. Subfamily consists of a single genus, *Aotus*. Numerous species have been described, but the validity of many of them as separate species is open to question. Nocturnal and arboreal; sleep during the day in well protected tree holes and move about freely in the trees at night. Diet consists chiefly of insects supplemented with fruit. Small rounded animals with soft fur of variable length; species from higher altitudes tend to exhibit a long pelage. Head rounded, face small and short with delicate features; eyes large, round and very prominent, pupils very large when dark adapted. Ears small, almost hidden in the long fur. Tail bushy and somewhat longer than body.

Aotus trivirgatus (Humboldt) (Fig. 39)

Very widely distributed in the Amazon and Orinoco basins. Full geographic distribution depends upon the validity of the various species. If the closely related infulatus, nigripes, senex, vociferans, oseryi, gularis, spixi, micronax and nigriceps are included with trivirgatus (Tate, 1939), then the distribution takes in a great region east of the Andes in Venezuela, the Guianas, Colombia, Brazil, Ecuador and Peru. Type locality, Casiquiare region of Venezuela. Total adult length 65 cm, of which somewhat over half is tail length. Coloration generally dark gray with some variation due to white hair tips. Three dark bands over the head from supraorbital ridges to occiput. Light spot over each eye varying from white to buff. Back dark, under parts light in color with differing proportions of gray, buff and yellow.

N. C. Davis (1931*b*) experimented with one *A. trivirgatus*, infecting it with aegypti mosquitoes carrying the Asibi strain of virus. There was no evi dence of any reaction, but the postexperimental blood serum was fully pro tective in a rhesus monkey protection test while the pre experimental serum was negative. Davis considered the monkey capable of being infected, but lack of animals precluded further experimentation.

The first comprehensive experiments with trivirgatus were made b Whitman, who inoculated four animals with Asibi virus. All four had ci culating virus in high titer; two died and two survived. The survivors ga clearly positive postexperimental mouse protection tests, indicative of i munity. A fifth monkey was infected by bite of aegypti mosquitoes carry Asibi virus. This animal became fatally ill and showed in abundance circulating virus at the time of death on the 13th day. The liver lesion the animals that died varied in severity but resembled those encount



Fig 39 *Aotus trivirgatus* (night mon
key)



41 *Cebus capucinus* (capuchin
key)





Fig 40 *Ateles ater* (spider monkey)

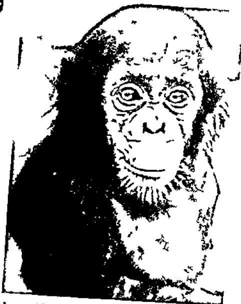


Fig 43 *Pan troglodytes troglodytes* (chimpanzee)

Fig 42 (Left) *Caluromys laniger* (woolly opossum)

the rhesus and saimiri monkey, there being a massive coagulation ne-
osis without striking zonal predilection.

In Colombia Bates and Roca García (1945) interposed four *A. trivirgatus* in transmission cycles with haemagogus mosquitoes and saimiri monkeys using the Rodas strain of virus. All four animals developed fever of 1 to 2°C and very high levels of circulating virus, comparable to the levels reached with Asibi virus in the rhesus monkey. All the monkeys died of the disease on the 4th or 5th day after infection. At autopsy, three of the four exhibited gastric hemorrhages. All four livers showed widespread coagulative necrosis and abundant Councilman body formation, consistent with the pathologic changes found by Whitman.

Subfamily *Cebinae*

Genus *Ateles*

[Elliot (1913) stated that the proper spelling, contrary to E. Geoffroy, was *Ateleus*. However, according to Tate (1939), the International Commission of Zoological Nomenclature accepts the form *Ateles*; hence *Ateles* is used here.] Spider monkeys. Body lightly and slenderly built; limbs and tail very long and slender. One of the most highly specialized arboreal primates; moves swiftly and gracefully through the forest canopy. Timid; usually found only in remote and undisturbed sections. Gentle; adjusts readily to captivity. Diet consists mainly of large quantities of fruits and leaves. Head rounded with protruding maxilla. Thumb small; in some species absent; in others... Tail prehensile and very highly specialized, serving as a fifth hand. Fur coarse, straight, often rough, especially over the head. Coloration black to brown.

Ateles ater (F. Cuvier) (Fig. 40)

Black spider monkey. There seems little doubt that the spider monkeys of the Guianas and of the Colombian and Venezuelan llanos are all of this one species. Type locality: Cayenne, French Guiana.

Experimental study with *A. ater* has been limited. N. C. Davis (1930) was able to obtain four of these animals, three of which were given As[ibi] virus by inoculation, while a fourth was bitten by aegypti mosquitoes infected with the S.R. strain. From this last monkey, and from one of the group infected with the Asibi strain, transmission was obtained to rh[esus] monkeys by bite of aegypti mosquitoes. The mosquitoes had been ...s on the 4th and 5th postinfection days, indic...

that the animals were circulating virus in effective amounts on those d

The animal with the S R strain of virus died of yellow fever on the 1 postinfection day Autopsy revealed slight icterus and cloudy swelling of liver and kidneys it appeared that the chief cause of death was the pare chymatous lesion of the kidneys The Asibi inoculated animal that infect mosquitoes on the 4th and 5th days was very ill on the 9th day and w sacrificed The liver appeared uninjured but the kidneys showed a mode ate cloudy swelling Liver emulsion was shown to contain yellow fever viru by transfer to a rhesus monkey Another Asibi inoculated monkey which showed no febrile reaction was bled 14 days after inoculation and found to have circulating antibodies by a rhesus protection test It was concluded that *A ater* was highly susceptible to yellow fever virus

At Villavicencio Colombia a number of sera from *A ater* were collected in the course of surveys for yellow fever immunity In the mouse protection tests these sera appeared to behave like those of the rhesus monkey showing either clear negativity or strong protection Since the entire population of *A ater* occurs within the known endemic yellow fever zone it is impossible to make an accurate statement about the frequency of nonspecific reactions However because of the high degree of parallelism with other closely asso ciated genera in this respect it may be assumed that such reactions are practically nonexistent if the sera are properly handled

Lloyd and Penna (1933b) inoculated *Ateles paniscus* (L) and *Ateles varie gatus* (Wagner) intracerebrally with neurotropic virus and observed fatal encephalitis Passage of the virus by brain emulsion was obtained Virus appeared to be limited to the central nervous system where the pathology produced was similar to that found in other genera

Genus *Lagothrix*

Lagothrix lagothrica (Humboldt)

Woolly monkey Originally described from the Guaviare River one of the main Colombian affluents of the Orinoco found throughout the forests of the Colombian and Venezuelan llanos usually in bands of 15 to 30 individ uals and in relatively isolated and unmolested areas Diet consists almost entirely of fruits and leaves Highly arboreal (has never been encountered on the ground) placid retiring gentle and while delicate and difficult to maintain in captivity readily tamed and affectionate Heavy with massive shoulders and powerful arms Face black nearly naked ... human in appearance and expression

hairs that give it the fleece like character from which the monkey derives its common name body hair black variably intermingled with gray especially dorsally Tail prehensile and powerful resembling tail of spider monkeys in its functional versatility *Lagothrica is the only member of the genus Lagothrix* that has been studied from the viewpoint of its association with yellow fever

Although this species was undoubtedly considered by Thomas (1907) in his work at Manaus the records were lost in the fire that destroyed the laboratory after Dr Thomas death The first published study of lagothrix with respect to its susceptibility to yellow fever was that of Davis (1930c) who infected 12 of these animals with the Asibi strain of yellow fever virus by means of either direct inoculation or aegypti transmission Most of the monkeys died although not of yellow fever However since the rhesus monkey protection test revealed protective antibodies in three of the four animals that survived long enough for immunity tests to be made it was concluded that lagothrix could become infected and could develop immunity With neurotropic yellow fever virus and intracerebral inoculation the monkeys developed a severe encephalitis and died but no visceral lesions were present (Lloyd and Penna 1933*b*)

Bugher inoculated a small number of lagothrix at Villavicencio Colombia with the Asibi strain with results similar to those of Davis There was no mortality directly attributable to the virus but the monkeys did show circulating virus in moderate amount Mortality resulting from laboratory confinement was always so high that it was difficult to keep the monkeys alive long enough to develop antibodies The performance of the sera in the mouse protection test however, was similar to that of the other primates

Genus *Cebus*

Capuchin monkeys [Taxonomy of the genus *Cebus* has not been agreed upon *Many species have been named mostly on the basis of color* variations which may be inconstant *All* species closely related The genus was revised by Elliot (1913) and further discussed by Tate (1939) Species names in this discussion follow usage of the author cited] Distribution includes Colombia part of the Guianas and Venezuela with extensions into Brazil Ecuador Panama and Peru Various species and races all fall into two broad groups based upon presence or absence of a crest which is most prominent in males In Colombia east of the Andes occurs the group represented by *Cebus fatuellus* Fatuellus is dark brown with black hands and feet Sides

of face and head grayish white. Tail prehensile and dark brown to black distally. Older individuals, especially males, have crest of two black temporal tufts hornlike in appearance. The second type, uncrested and generally light faced, is represented by *Cebus capucinus* (Fig. 41). Head and face white or yellowish white, rest of body dark brown or black. Total adult length about 100 cm., of which tail is about one half.

The first experiments of which we have records available were performed by Aragão (1928), who inoculated two *Pseudocebus azarae* (later called *Cebus azarae*) with Asibi virus. One showed no reaction; the other had fever and was sacrificed, but no lesions could be found. The following year N. C. Davis and Shannon (1929a) infected *Cebus macrocephalus* with the Asibi, B.B., and F.W. strains of virus, using infected *A. aegypti* to transfer the virus. They were able to infect fresh lots of mosquitoes on these monkeys and to secure transmission to other cebus monkeys. In addition, Davis and Shannon made passages of the virus by inoculation to other cebus and to rhesus monkeys. The infected cebus did not develop clinical or pathologic signs of yellow fever and upon external examination appeared refractory. However, the presence of circulating virus on several days was demonstrated both by the ability of aegypti to become infected by blood meal and by transfers of serum to rhesus monkeys.

Cebus albifrons and *Cebus frontatus* were studied by Davis (1930a) using the Asibi strain and passing the virus from monkey to monkey both by inoculation of serum and through the agency of *A. aegypti*. Some of the animals showed fever and signs of slight illness, in at least one instance death with liver necrosis occurred and was considered due to yellow fever. Circulating virus was demonstrated several times between the 3d and 8th postinoculation days, and it was found that serum from convalescent animals gave protection to rhesus monkeys. In addition, it was shown that animals that had recovered from an infection were completely resistant to further introduction of virus.

Davis (1931b) continued his experiments, this time with *Cebus variegatus*, using the Asibi and S.R. strains and again infecting the monkeys with *A. aegypti*. The animals had circulating virus, as shown by their ability to infect fresh mosquitoes; they were shown to produce antibodies by the rhesus monkey protection test.

Lloyd and Penna (1933b) produced fatal encephalitis in *C. variegatus* and in *C. frontatus* by direct intracerebral inoculation of neurotropic virus.

Passages with filtered brain suspensions failed occasionally because of virus loss in filtration

At Villavicencio, Colombia, a small number of *C. fatuellus* were inoculated with Asibi virus by Bugher and his coworkers and were found to circulate the agent in moderate titers (10^{-2} to 10^{-3}). The animals showed no signs of illness, and after 30 days the mouse protection test was strongly positive. The immunologic reactions were clear cut and there was no evidence of nonspecificity. It was noted that the undiluted serum was highly toxic to white mice on intracerebral inoculation and that this toxicity disappeared on diluting the serum 1:2.

Five complete cycles with *A. aegypti*, *Cebus versutus*, and the J.Z. strain of virus were maintained without difficulty by Waddell and Taylor (1945). The J.Z. strain was used because it seemed to be more virulent than others for cebus monkeys. All 13 monkeys used had circulating virus from the 3d to the 6th day; in five monkeys virus persisted until the 7th day. The maximum titer was less than 10^{-4} in some instances and only rarely reached 10^{-6}.

Waddell and Taylor next tried the Colombian Volcanes strain, which had a relatively low order of virulence for cebus. Successful transmission cycles were obtained but with some difficulty, apparently because of low mosquito infection rates associated with low virus concentrations in the blood stream.

In the light of the demonstrated importance of variables such as virus strain, the failure of Bates and Roca Garcia (1946*a*) to obtain transmission with haemagogus mosquitoes and Perez virus cannot be construed as a fundamental difference in susceptibility between the cebus of the region of Villavicencio and the Brazilian species. Similarly, there is no basis for concluding that this monkey is of minor importance in yellow fever epidemiology. The survey indicates that cebus become infected with as high a frequency as other monkeys in yellow fever regions. Also, it should be remembered that in spite of the diversity of opinion with regard to cebus species names, there are apparently no real differences in yellow fever susceptibility among the various members of the genus.

MARSUPIALIA

The marsupials constitute a very populous and complex order in South America. The evidence concerning their place in the epidemiology of jungle yellow fever is variable and, in some instances, contradictory. Marsupials

like monkeys, occur both within and without the known jungle yellow fever area. The evidence implicating the marsupials in yellow fever epidemiology is essentially similar to the evidence with respect to the primates, but there are certain quantitative differences that complicate the situation somewhat.

All members of the order in South America are predominantly nocturnal and arboreal. They feed chiefly at night and are comparatively indiscriminating in their food—they eat birds' eggs and young birds, grubs, worms, insects, and whatever fruit may be available; leaves do not appear to constitute an important component of the diet. They usually sleep during the day time, and most of them are casual in their sleeping habits: a limb or the fork of a tree serves for a resting place as readily as tree holes or hollow trunks. Contrary to most species of monkeys, the marsupials adapt themselves very well to human invasion of the forests and appear to flourish in areas of second growth where there is considerable agriculture. They bear two large litters a year, so that there is a rapid turnover of population and the relative preponderance of species may alter rapidly as conditions in an area change. All of the important South American marsupials belong to the family *Didelphidae*. There is some difference of opinion concerning the taxonomic position and exact nomenclature of the various species, but in this discussion the terminology suggested by Gilmore (in Bugher, Boshell-Manrique, et al., 1941) will be used.

Didelphis marsupialis (L.)

Common opossum. Extends from southern Mexico throughout northern South America to central Argentina with the exception of high altitudes in the Andes and central and southern Brazil. Intergrades in Mexico (*Didelphis aurita*) with *Didelphis virginiana* of the United States, the differences being essentially in color pattern; hence *D. marsupialis* and *D. virginiana* may be considered together. Ears black in *D. marsupialis* but may be tipped with white in *D. virginiana*. (Young of *D. marsupialis* white-eared until about 4 months of age (1 month out of pouch) (Bugher, Boshell-Manrique, et al., 1941) and may therefore be confused with *Didelphis paraguayensis*.) Body weight varies from 1 to 5 kg; body length from 60 to 80 cm. Color grizzled black and white; coat consists of long guard hairs mixed with short soft underfur. Tail long, nearly naked ventrally and lightly haired dorsally.

The first studies of this species with respect to susceptibility to yellow fever virus were made by Whitman in Brazil. He inoculated four animals

with Asibi virus and then bled them at intervals, testing the sera for circulating virus by intracerebral injections into mice. He found that one animal circulated relatively small amounts of virus without signs of illness. Two to 3 weeks later antibodies could be demonstrated in the serum of this opossum and in the serum of one other by means of the mouse protection test. Whitman noted at the time that the neutralization reactions of these animals were relatively weak.

Little further interest in the species appears to have been displayed until the systematic studies of marsupials at Villavicencio, Colombia (Bugher, Boshell-Manrique, et al., 1941). Interest in the opossums was stimulated by their occurrence in large numbers throughout the yellow fever area and especially by their position as the dominant mammal in Muzo, Boyacá, long known as a region of endemic yellow fever (Franco, Martínez Santamaría, and Toro Villa, 1911; Kerr and Patiño Camargo, 1933). The tests employed small doses of viscerotropic virus administered chiefly by the subcutaneous route. The Martínez strain of virus was used in all except a few instances where Asibi virus was employed. The animals were tested daily for circulating virus. Serum taken before the start of the experiments was stored until the serum taken 1 month after inoculation was available, and then both specimens were tested for the presence of antibodies in the same protection test run. This practice avoided pre-experimental selection of animals on the basis of serologic behavior and permitted correlation of protection test results with response to the virus, one of the chief objectives of the studies.

Following virus inoculation there were no signs of illness or other physical manifestations of infection. The numerous deaths in the series all appeared to be due to causes other than the virus and especially to the multiple cardiac punctures for blood specimens to which the animals were subjected. There were no pathologic changes attributable directly to yellow fever. A total of 119 animals that proved to have negative pre-experimental protection tests received virus doses of from 100 to 1,200 ID_{50}. Titrations for circulating virus were carried out in 56 of these, and six animals showed circulating virus variously from the 5th to the 9th postinoculation days, with the maximum titer in the range of 10 [illegible]. Of the original 119 animals 94 (including two of the six that had shown circulating virus) survived for 30 days to be tested for antibodies by the mouse protection test. 19 (again including the two known to have circulated virus) of these 94 gave positive reactions. Thus only 20 per cent of animals with negative protection tests were made positive by direct inoculation of virus, while wild-caught animals

from an area of recent yellow fever showed as high as 33 per cent positives

The first question to be raised was naturally the specificity of these reactions *D marsupialis* were accordingly trapped from high altitudes where yellow fever has not occurred within known times To these was added a group consisting of animals born in the screened normal animal house where the probability of virus contact would be practically zero The total was 93 animals all of which gave protection test reactions interpreted as negative In addition 116 young adults and suckling young from the Villavicencio region all born since the last period of known yellow fever in the area were tested and all gave negative protection tests Therefore it was concluded that nonspecific protection test reactions in this species are negligible provided that sound standards of interpretation are used (Bugher 1940) and that reasonable care is employed in handling the sera (It was found that normal serum of the genus *Didelphis* produces a constant but relatively slight nonspecific inactivation of yellow fever virus This reaction is similar to that of normal human serum when compared to that of the rhesus monkey but is somewhat more severe It is not a matter that in practice appears to give significant difficulty)

TABLE 10

DIDELPHIS MARSUPIALIS FREQUENCY OF CIRCULATING VIRUS FOLLOWING INOCULATION OF SMALL DOSES OF VARIOUS STRAINS OF YELLOW FEVER VIRUS

Virus strain	*Circulating virus*		*Total*
	+	−	
Volcanes	0	4	4
Asibi	0	3	3
Chichimene	15	10	25
Ilab	0	3	3
Gordillo	0	4	4
Tamborredondo	1	3	4
Total	16	27	43

\+ = circulating virus positive 1 or more days
− = circulating virus negative for all bleedings.

More sensitive protection tests were devised but even the most sensitive of these modifications failed to abolish the discrepancy between field observation and laboratory experiment nor did the information available at

that time explain the conflicting facts. The possible effect of strain differences was considered, but there was no evidence that this might be a factor until C. R. Anderson investigated several different strains in order to see whether any one strain would yield a better proportion of animals circulating virus. Using small doses he obtained the results shown in Table 10. The high incidence of circulating virus with the Chichimene strain was noted at once and this strain was thereafter used in Anderson's experiments, hence the disproportionate number of animals inoculated with this virus.

Following this, *D. marsupialis* from Rio de Janeiro, Minas Gerais and Espirito Santo were studied by Laemmert (1946), using various strains of yellow fever virus and animals of different ages. He employed a total of 162 adult, juvenile, and newborn animals, all of which came from a yellow fever area and all of which gave clearly negative results in a pre-experimental mouse protection test. Of 82 adults, only one (belonging to a group of 18 inoculated with the Martinez strain) circulated virus, only three of the 82 developed neutralizing antibodies. Among the 27 juvenile animals 6 of the 21 inoculated with a small dose of the Asibi strain were the only ones to develop antibodies. Among the newborn, the only animals that developed antibodies were four of the 13 that received the J/ strain.

Laemmert's results indicated that there was a significant difference between strains of virus with this species, but even more important, he showed that *D. marsupialis* in Brazil was resistant to infection with various strains of yellow fever virus. He concluded that the species could not play a part in the epidemiology of jungle yellow fever in that area of Brazil, a conclusion later supported by the investigations at Ilheus (Laemmert, de Castro Ferreira and Taylor, 1946) where none of 227 *D. marsupialis* captured in an active yellow fever area gave evidence of circulating antibodies.

That this conclusion does not hold for all of Brazil was shown by Whitman (1943) in his studies on the young mouse protection test. Negative results were obtained with all of 23 sera from the Federal District, considered to have been free of yellow fever for several years, however, 50 specimens from Bem Posta, where there had been yellow fever within a year of the collections, gave 16 positives, although the same 50 sera had previously given uniformly negative results with the less sensitive routine intraperitoneal test.

Further information on the matter of uniformity of susceptibility of this species is furnished by Theiler, who studied the North American *D. virginiana*. This species, as mentioned earlier, may be considered the most

northerly variant of *D. marsupialis*. Using the Asibi strain of virus in small doses (approximately 150 LD$_{50}$), Theiler tested 17 animals. His findings conformed very closely to the results obtained by Bugher, Boshell-Manrique et al. (1941) with *D. marsupialis*, both with respect to the frequency of circulating virus and to the immunologic response. If the North American distribution is considered simply as the extension of *D. marsupialis* from South America by way of Central America, then concordance of the results in Colombia with those in the United States is not surprising. The Brazilian coastal distribution would then be relatively more remote and might well constitute a different population. From this point of view there would appear to be evidence in this species of definite geographic variability in susceptibility to yellow fever virus, presumably on a genetic basis.

The immunologic response to virus infection in the species appears to be entirely consistent. As noted by Bugher, Boshell-Manrique et al. (1941) and confirmed by Theiler and by Anderson, there is a slight virus inactivation by the serum of normal *D. marsupialis*; the effect is greater than shown by human sera and less than that commonly found in pigs and peccaries (Bugher, 1940), and in practice does not occasion difficulty in the interpretation of protection tests with this species. Despite the statements that false positives are commonly obtained, Bates (1944a) did not substantiate the claims, and Laemmert (1946) did not obtain a single positive among 227 *D. marsupialis* tested. The type of false positive that may be expected to occur occasionally is due to adventitious factors or to extraneous matter introduced into the sera in the course of manipulation during the test. Since the problem seems to be concerned with the test itself and not with intrinsic properties of the sera, the answer lies in retesting the questionable sera.

False positives are rare with *D. marsupialis* and their occurrence with any appreciable frequency may be taken as an indication of unsatisfactory test procedure. The greatest problem with *D. marsupialis* is that of false negatives. As all who have worked with these animals have recognized, the antibody response to infection is not only generally feeble but also highly variable; therefore it is essential to maintain test sensitivity at a level that will demonstrate the virus-neutralizing capacity of weak sera. Otherwise, animals that have in fact been infected and are immune will be erroneously classified as negative on the serologic evidence.

In summary, *D. marsupialis* exhibits a relatively low order of susceptibility markedly influenced by the strain of virus concerned. The circulating virus levels are low but of an order capable of infecting some mosquitoes. The

antibody production is relatively weak and variable, probably with a tendency to become weaker with time (Bugher, Boshell Manrique, et al 1941) consequently an appreciable frequency of negative interpretations may be expected among animals that are actually immune to small doses of virus While there is a slight nonspecific virus inactivating property in the serum of this species nonspecific positive protection tests result almost entirely from defective tests and not from properties of the sera The epidemiologic evidence with regard to *D marsupialis* is difficult to evaluate The extent to which the species may be involved in the yellow fever picture of a particular area can be determined only through weighing as a whole all the data pertaining to the region in question While it does not follow that an animal successfully infected under natural conditions is automatically capable of infecting mosquitoes that may feed upon it to reject clear evidence of such infection on the vague grounds of false reactions is to be guilty of selecting facts to fit a preconceived theory

Didelphis paraguayensis (Oken)

White eared opossum Found in higher levels of the Andes from Venezuela to Bolivia (2 000 m) and over the highlands of central and southern Brazil Face marked with median and ocular stripes Smaller than *D marsupialis* weighs from 0 5 to 2 kg measures 50 to 70 cm over all

Bugher Boshell Manrique et al (1941) tested 16 of these animals fro country known to be free of yellow fever using a very small dose of th Martínez strain of virus No circulating virus was recovered but neutralizin antibodies were detectable in three of 11 animals that survived long enou to be tested All of the animals had given clearly negative protection t at the time of capture and there was no evidence of nonspecific protect test behavior Laemmert (1946) inoculated eight Brazilian *D paraguaya* with Asibi J Z and O C strains and tested for circulating virus on th and 7th days One animal with the Asibi strain showed a small amou virus on the 2d day and developed antibodies another also with A showed no circulating virus in the two bleedings but did develop bodies The other six showed neither circulating virus nor antibodies

The species appears to be less susceptible than *D marsupialis* al further investigation with other strains of virus might reveal a mark ference in susceptibility depending on the strain In any case the ographic range takes it out of the endemic yellow fever zone

Philander (= Metachirops) oposum (L.)

Gray masked or four-eyed opossum with pouch; in Colombia called *chucha real*. Color dark gray with slight admixture of white; two large white spots over eyes. Total adult length 50 to 60 cm, with the tail slightly more than one half of this. Tail haired basally for about 5 cm, remainder bare, black in color with white tip.

This species was relatively abundant in the region of Villavicencio where the first studies were made by Bugher, Boshell-Manrique, et al. (1941). A total of 26 animals was given small inoculations of the Asibi and Martinez strains and bled on the 5th and 7th days for circulating virus tests. Of the 15 animals whose pre-experimental sera proved negative with the protection test (the pre-experimental sera were not tested until the postexperimental sera were also available) three showed circulating virus; two of the three belonged to a group of six inoculated with the Asibi strain. Protection tests 30 days following inoculation demonstrated circulating antibodies in these three but not in any of the others. Of the 11 animals that turned out to have naturally occurring positive protection tests, none showed virus circulation or any significant change in serologic reaction.

Bates (1944a) extended the series, using 28 animals and various strains of virus. He found one animal with a small amount of circulating virus on the 5th day. This animal gave a clearly positive pre-experimental protection test; it was impossible to obtain a postexperimental serum because the animal died very early.

Adding Bugher's data to his own, Bates concluded that the protection test results were entirely random and that the relationship of circulating virus to the pre-experimental protection tests was one of pure chance. On the basis of the combined data the probability of selecting a sample of 11 on some arbitrary basis and having them fail to show circulating virus would be of the order of 0.12, clearly in agreement with Bates' conclusion. Obviously the interpretation of the results is intimately connected with uniformity of technique in the manipulation of the sera and in the conduct of the tests. While it seems probable that most discrepancies with philander are technical in origin, the question cannot be definitely resolved without further careful study of the matter. There is as yet no evidence of marked difference in susceptibility to various strains, but this matter also must be left open for further review in the light of the developments with some of the other genera. For the present it seems justifiable to consider that

philander like *D. paraguayensis* is unlikely to be of significance in the epidemiology of yellow fever.

Metachirus nudicaudatus

Brown masked or four-eyed opossum. Nudicaudatus is the only example of the genus *Metachirus*. Type specimen described from Caquetá, Colombia; widely distributed in northern South America east of the Andes; one of the important mammals of the Muzo region. Superficially closely resembles *P. opossum* in general size and in presence of two light-colored spots above the eyes. Usually has no pouch but rudimentary pouch may occur (Gilmore in Bugher, Boshell-Manrique, et al., 1941). Fur soft and silky, dark brown dorsally, becoming much lighter ventrally. Tail naked for distal two-thirds, hence the name nudicaudatus.

The original studies (Bugher, Boshell-Manrique, et al., 1941) were made with Martinez virus on a sample of seven, all of which had negative protection tests at the time of capture. It was quickly evident that this species was more uniformly susceptible than any other marsupial of the Villavicencio area, five of the seven animals inoculated showing circulating virus in titers of approximately $10^{-[illegible]}$. Four, including one of those which had failed to show circulating virus, survived for a postexperimental protection test; three gave positive results, while the one that had not exhibited circulating virus gave a negative test. The antibody response appeared to be similar to that of the other marsupials studied in that it was relatively weak compared with the primates but readily demonstrable with a reasonably sensitive test. There was no indication of nonspecific reactions. As in the other marsupials studied the virus infection was not associated with visible illness, and although mo tality from the bleedings was high, none of the deaths appeared to be relat to the yellow fever infection.

Because of the greater susceptibility of this species to yellow fever vir the studies were continued by Bates (1944*c*), who made a successful serie 10 passes of Novoa virus through nudicaudatus. Part of the series was c ducted with desiccated serum whose virus content was determined at e pass, and the remainder by blind passes, that is to say, with fresh se of unknown virulence. No great difficulty was encountered in maint this series, in marked contrast to the similar attempts with *D. marsup* which had failed. There was no evidence that the passes had in an modified the virus, nor were the titers in the later portions of the seri cantly higher than in the beginning.

Serologically the series extended considerably the basis for the evaluati
of protection test results although the virus dosage in the tests vari
through a greater range than is desirable A total of 35 animals received si
nificant doses of the virus the number surviving long enough for pos
experimental protection tests was 20 among which were 12 of the 21 tha
showed circulating virus and 8 of the 14 that did not show it The serology
of the 20 survivors is set forth in Table 11

TABLE 11

METACHIRUS NUDICAUDATUS ANTIBODY RESPONSE TO YELLOW FEVER VIR
POSTEXPERIMENTAL PROTECTION TESTS

Pre-experimental protection test status	Animals with circulating virus			Animals without circulating virus		
	Positive	Negative	Total	Positive	Negative	Total
Positive	1	0	1	1	0	1
Negative	8	3	11	2	5	[illegible]
Total	9	3	12	3	5	8

One striking inconsistency is the animal with a positive pre experimental
protection test that showed circulating virus but it seems likely that in this
case a technical error crept in Another inconsistent finding lies in the ani
mals that failed to show antibodies after exhibiting circulating virus The
origin of these few contradictions cannot be stated with certainty but in
view of the studies of Waddell and Taylor (1948) it is probable that insuffi
cient time was allowed before the final bleedings

Unfortunately the frequent occurrence of false positives in this and other
species of Colombian marsupials has erroneously been inferred by Laemmert
(1946) and by others on the basis of Bates statements of his results in the
above experiment It seems necessary to emphasize that with neither meta
chirus nor didelphis nor caluromys has there been any indication that
marked nonspecific virus neutralizing capacity of the sera is anything but
very rare Most of the conflicting data would appear to arise from failure to
distinguish animals that have had prior infections from genuinely suscep
tible animals and this is chiefly a matter of test sensitivity and technique

The outstanding feature of the behavior of M nudicaudatus particularly

in view of its high susceptibility to the virus has been the lack of evidence of previous infection among the species in the Villavicencio area Only one clearly positive protection test has been observed and this in an animal also recorded as showing circulating virus on subsequent inoculation However most of the metachirus studied at Villavicencio were captured in valleys where active yellow fever had not been demonstrated and thus the population may have escaped exposure to the virus

Waddell and Taylor (1948) working with aegypti mosquitoes and the O C strain of virus were able to establish a long series of cyclic passages through metachirus by initially alternating metachirus and marmosets in the mosquito passages after which they were able to continue in metachirus alone The Almada strain (Taylor and Fonseca da Cunha 1946) was transmitted readily by *A aegypti* without the interposition of marmosets Approximately 60 per cent of the mosquitoes used for transmission of Almada virus were found infectious by bite, as compared with 10 per cent or less in a parallel experiment with the O C strain

M nudicaudatus was restudied by Bates and Roca Garcia (1946a) using infected *Haemagogus capricornii* (= *H spegazzinii*) to introduce the virus by bite Of 13 animals exposed to the bites of the mosquitoes 11 developed circulating virus as shown by daily bleedings from the 3d to the 7th days Circulating virus did not occur beyond the 5th day The number of mosquitoes biting each animal varied from one to five Fresh haemagogus mosquitoes were successfully infected on the metachirus and a full cycle of transmission demonstrated Bates and Roca Garcia noted that a lower proportion of the mosquitoes became infected than when an aotus or saimiri monkey was used probably a reflection of the lower virus levels encountered in metachirus

Considerable light was thrown on the rate of development of antibodies in metachirus by Waddell and Taylor (1948) who conducted infection experiments with the O C strain of yellow fever virus in the Brazilian *M nudicaudatus* considered closely related to or identical with the type form from Colombia These are the most extensive studies yet made on metachirus Over 50 animals were used in the series A mouse survival ratio of 4/6 was taken as the criterion of a positive protection test All of 53 known infected metachirus eventually developed antibodies although 3 weeks after infection eight were still negative of these eight two became positive at about 5 weeks three at 2 months and the final three by the time 3 months had elapsed It was noted that these eight sera although they were considered

negative in the early postinfection period gave considerably greater aver survival times (A S T) than the negative preinfection sera

Of especial interest were the findings with respect to the disappearance of the demonstrable antibodies Of 43 animals with positive postinfection protection tests 11 (22 per cent) had negative tests 3 months later but no further change was demonstrated during the year that the observations were continued However it was noted that the 11 sera considered negative permitted abnormally long survival times in the test mice This marked drop in antibody titer within 3 months after infection in an appreciable fraction of the experimental animals together with unchanged antibody levels in the majority is substantially in accord with the results obtained by Anderson and Roca Garcia (1947) in caluromys In Waddell and Taylor s experiments in the immediate postinfection bleedings the errors were in the direction of failing to demonstrate antibodies rather than in the occurrence of false positives This is in close agreement with the general conclusion of Bugher Boshell Manrique et al (1944) that the protection test does not differentiate all of the animals that have had previous infections with the virus In properly conducted tests the positives are highly indicative of prior infections but the negatives may constitute a mixed immune and non immune population

M nudicaudatus appears to be the marsupial most consistently susceptible to the virus of yellow fever with the possible exception of some of the marmosa The species seems quite uniform in this respect and does not manifest the geographic variation encountered with *D marsupialis* Virus levels are moderately high with some strains of yellow fever virus and with these mosquitoes may be infected readily and transmission cycles maintained under experimental conditions As with the other marsupials the animals themselves are not visibly affected by the presence of the virus and appear to engage in a normal amount of movement and activity Serologically false positives do not occur with any significant frequency but many of the postinfection sera show weak virus neutralization and will give negative results in an insensitive test There is considerable variation not only in the amount of antibody formed but also in the time at which it may reach a maximal value An appreciable fraction of the actively immunized animals have a decrease in their neutralizing reactions within 3 months and may give reactions interpreted as negative

Caluromys (Mallodelphis) laniger (Fig 12)

Woolly opossum South America east of the Andes from Colombia to Bolivia and east to the mid Amazon and central Brazil a related species *Caluromys derbiana* occurs in western Colombia Color reddish brown pelage long and distinctly woolly Tail long (30 to 40 cm) haired dorsally for about three quarters of its length and ventrally for about one third Ears dark violaceous (black in prepared specimens)

Three of these animals all with negative pre experimental protection tests were investigated by Bugher Boshell Manrique et al (1941) using Martínez virus Two were tested for circulating virus with clearly positive results The third animal which was not bled for circulating virus determinations was the only one to survive for a postexperimental protection test at 30 days it gave a strongly positive reaction It was concluded that this species had a susceptibility comparable to that of *M nudicaudatus*

Anderson and Roca García (1947) greatly extended the knowledge of the reaction of *C laniger* to yellow fever virus by studying 100 specimens from three different regions of Colombia with various strains The greatest differences found with respect to strain of virus were between the Volcanes and Tamborredondo strains two of nine animals circulated virus with the former strain in contrast to all of 8 with the latter Most of the animals however were inoculated with the Chichimene strain out of 56 animals 46 showed circulating virus Virus was encountered variously from the 2d through the 10th day Titers of circulating virus were determined in a number of instances and found to be low the highest being 10^{3} The great majority of the titers were in the neighborhood of 10^{1} or less

There were no discernible signs of illness due to the virus infections in these animals and while mortality was moderately high during the course of the experiments the deaths did not appear to bear any relation to the yellow fever infection but rather were connected with the trauma of the bleedings intercurrent infections and parasitism

Especial attention was paid to the immunologic behavior of the animals both by studying the production and duration of neutralizing antibodies and by observing the response of previously inoculated individuals to large challenge doses of virus Table 12 gives the protection test survival ratio information for 48 animals that circulated virus and survived for a post experimental bleeding (Since no animal with a preinoculation survival ratio of 2/6 or greater ever circulated virus a ratio of 2/6 was accepted as

evidence of antibodies The preinoculation and postinoculation sera of a given animal were tested in the same run to equalize variable factors The authors data have been grouped somewhat to save space)

TABLE 12

The Distribution of Neutralization Test Survival Ratios of Preinoculation and Postinoculation Sera from Infected C laniger

Type of serum	*Neutralization test survival ratios*							
	0/6	1/6	2/6	3/6	4/6	5/6	6/6	*Total*
Preinoculation	42	6						48
Postinoculation		3	3	5	10	14	1	48

As can be seen the postinfection survival rates like those with most marsupials were highly variable and generally indicative of weak antibody response However duplicate and triplicate tests on the sera with the lower survival ratios gave highly reproducible results especially when N S I criteria were used In addition no nonspecific reactions were encountered in the entire series despite the high level of sensitivity employed in the tests

Especially significant were the studies made on *C laniger* to determine the extent to which neutralizing antibodies are maintained Most of the animals had positive protection tests within 14 days following the infecting dose but a few showed their maximum response only after 30 days Some of the animals survived over a year It was found that either an animal maintained its antibody at a constant level or began to lose it soon after the experiment and continued to lose it steadily until the protection test became negative Once lost detectable neutralizing antibody was never regained save after a second injection of virus There were no spontaneously developing positives either among the normal unused animals or among once positive animals that had become negative There was no correlation between loss of demonstrable antibody and any factor in the infection such as infecting dose amount of circulating virus or original level of antibody

The Challenge of Previously Inoculated Woolly Opossums

Thirty two animals which had received prior inoculations of virus and which had reacted in various ways were reinoculated at varying intervals

with moderately large doses of the Chichimene strain. The majority reacted in accordance with accepted immunologic principles, even most of those that manifested a regression of the neutralization to a level interpreted as negative still retained an effective immunity. However, three animals showed multiplication of virus for a second time. Thus it appears that in some individuals of this species the loss of the neutralizing capacity of the serum may be associated with complete disappearance of immunity so that the animal is again susceptible.

Genus *Marmosa*

Murine opossums. An extensive and complicated genus, distinguished from other murine opossums by a long (12.5 to 22.5 cm.), bare tail and large ears. Marmosa range from southern Mexico to Argentina. Small, total adult length 20 to 40 cm., weight from 20 to 75 gm. Colors are soft grays and browns.

Very few marmosa were captured at Villavicencio, Colombia, although it is probable that they are far more numerous than would appear to be the case. Only one—not fully identified as to species but considered to be *Marmosa cinerea* (Bugher, Boshell-Manrique, et al., 1941)—was given an inoculation of yellow fever virus. This animal died 6 days after receiving a very small dose of the Martínez strain, and a large amount of virus was found postmortem in a suspension of the heart and liver combined.

Using aegypti mosquitoes and the Almada strain of virus, Waddell and Taylor (1948) established two transmission series with *M. cinerea*. The firs series was carried for three cycles and the second for four cycles befor termination due to failure of the mosquitoes to become infected. Virus circ lated in most of the animals for 2 days only, the titers reached being simil to those encountered with metachirus. During the period from the 2d to t 5th day following infection in the successful cycles *M. cinerea* was infect to 44 per cent of the mosquitoes fed upon it. All of the animals that survi the experiment developed neutralizing antibodies; two of 10 were nega at 1 month but became positive by the end of the 2d month.

General Summary of the Marsupials

The extensive investigations of the marsupials and their relation to y fever have revealed a consistent pattern of behavior. All appear susce to the virus in some degree, but the apparent susceptibility is modif factors among which the strain of virus used for testing is

important one. The studies on *D. paraguayensis* and *P. opossum* indicate a very low order of susceptibility for these two species. The genera *Metachirus*, *Caluromys*, and *Marmosa* have a higher order of susceptibility with particular virus strains, and with the genera *Metachirus* and *Marmosa* successful mosquito-marsupial transmission cycles have been maintained in the laboratory.

The immunologic behavior of the marsupials is highly consistent when neutralization tests are carefully made at a sensitive level. Nonspecific reactions interpreted as positives are rare and probably occur with about the same frequency as in primate sera (exceptions: philander and Koprowski's marmosa). With the serum of *D. marsupialis* there is a slight nonspecific inactivation of virus that is not apparent in the other genera and that gives no difficulty in interpretation. The immunologic response to virus is relatively weak throughout the order in comparison with the primates and is highly variable both in degree and time of development of the antibodies. Further, an appreciable fraction of metachirus and caluromys lose their neutralizing antibodies after a few months, and in the case of caluromys it is clear that some of such animals can be reinfected. However, most of the animals that lose their demonstrable antibodies do not appear to regain their susceptibility, so that the negatives of a wild population that has been exposed to yellow fever may form a mixed sample of susceptible and insusceptible animals. This was suggested by Bugher (1941) as a significant factor in the laboratory behavior of such animals.

A high incidence of test results presumed to be false positives has been reported by Koprowski (1946) on the basis of the neutralization of both yellow fever and Japanese B viruses. He postulated that no infectious agent giving rise to antibodies capable of neutralizing Japanese B virus exists in Brazil; therefore any reaction against Japanese B is nonspecific, and it may be presumed that the reaction of that serum with any other virus is also nonspecific. Since viruses closely related to Japanese B do occur in Asia, Africa, and North America, Koprowski's assumption that no such agent occurs in South America is obviously tenuous. Furthermore, Koprowski's findings are directly contradicted by Laemmert, de Castro Ferreira, and Taylor (1946), who found no positives at all in 227 *D. marsupialis* captured in Brazil, and also by Bugher, Boshell-Manrique, et al. (1941), who obtained only negative results with 193 animals from the Villavicencio region.

In the face of such conflicting evidence, it seems most reasonable to conclude that the nonspecific reactions resulted from the manner of collecting

the blood specimens and the technique of handling and preparing them for the testing rather than from some peculiar behavior characteristic of the animals themselves Bacterial contamination for instance is one very fruitful source of nonspecific positive protection tests in sera from normal animals of all kinds including primates With the smaller animals where it is difficult to obtain a sufficient volume of blood for testing it is generally recognized that the greatest care is necessary if frequent contaminations are to be avoided

RODENTIA

The rodents in South America constitute a very complex and extremely populous order which has been studied only in part with regard to its susceptibility to yellow fever virus There does not appear to be any systematic character to the susceptibility of members of the order so that it becomes necessary to consider certain species individually In general most species of rodents are relatively resistant to yellow fever save when the virus is inoculated into the central nervous system a route that is often of great experimental value but of no consequence from the epidemiologic point of view

The more common laboratory animals of this order such as the white mouse the guinea pig and the rabbit have already been treated in Chapter 2 and will not be further discussed here except to call attention to the importance of the genetic background of the varieties within the species Among both white mice and guinea pigs there are great variations in degree of susceptibility to yellow fever virus that are based upon genetic differences By selective breeding even within a highly inbred strain of white mice it is possible to develop either highly susceptible or moderately resistant strains (Sawyer and Lloyd 1931 Lynch and Hughes 1936) In considering the differences in susceptibility of various populations of a single species it is important to remember that selective factors may have been in operation While these differences may be geographic in distribution they may also occur with time within a particular area

In South America the rodents that are susceptible to subcutaneously inoculated yellow fever virus are either terrestrial or partially aquatic Because of this and because they are active either at night or during the early morning or late evening hours they tend not to share the habitat of haemagogus mosquitoes the chief forest vector during the peak biting hours of these insects

Cuniculus paca

Commonly called *paca* in Brazil and *lapa* in Colombia. Widely distributed throughout the Guianas, Venezuela, Colombia, and Brazil. One of the largest rodents, often attains a length of 60 cm and a weight of 12 kg. Exhibits considerable color variation, predominantly brown to reddish brown in lower altitudes, darker brown higher in the Andes. Subsists largely on roots and succulent shoots. Builds complex burrows with several entrances. Highly esteemed as a food animal.

Nine specimens of the Colombian *C. paca* were tested for susceptibility to small doses of either the Asibi or Martinez strain of yellow fever virus by Bugher and his associates. Nearly all showed circulating virus variously from the 3d to the 9th days. The level of circulating virus was never high, the maximum titer being in the region of 10 . As in the guinea pig, antibody production was readily demonstrable. One of the animals had a weakly positive pre-experimental neutralization test, confirmed by a repeat test of the same serum. This animal circulated virus and showed a normally positive protection test 30 days after the virus inoculation. It was concluded that the preinoculation serologic reaction was nonspecific and probably due to some extraneous factor introduced into the pre-experimental serum. No other such reaction was encountered in the wild-caught animals.

Laemmert (1948) studied *C. paca* from Brazil, finding that of six animals inoculated with one or another of three virus strains, all circulated virus and five developed neutralizing antibodies.

It was concluded that *C. paca* was susceptible to small doses of virus and reacted in a consistent immunologic manner, although there was no evidence of the species having been infected under natural conditions in the Villavicencio region. It is conceivable that in a different habitat, and especially with mosquitoes other than haemagogus acting as vectors, *C. paca* might play an active part in the epidemiology of yellow fever. No such evidence is available at present.

Genus *Dasyprocta*

Agoutis. Very widely distributed in Central and South America, very abundant throughout forested regions of northern South America in the lower altitudes. Dasyprocta fall into three groups (Tate, 1939); east of the Andes occur two of the groups, one brownish red and the other gray. In the Villavicencio region the gray form predominates and has been studied under the

name of *Dasyprocta variegata* Agoutis have characteristics intermediate between pacas and rabbits Smaller than pacas and have long powerful hind legs that enable them to travel with a bounding gallop when alarmed Ears relatively small head less broad and muzzle more pointed than in pacas Live in burrows and may also swim on occasion accustomed to move about both by day and by night Communal to some extent when females with litters are together young appear to suckle indiscriminately

Lloyd Penna and Mahaffy (1933) inoculated neurotropic yellow fever virus intracerebrally in Brazilian *Dasyprocta aguti* and observed clinical signs of encephalitis followed by death in most of the animals The virus could be recovered *in large amount from the central nervous system*

In 1938–1939 Bugher and his colleagues studied nearly 90 dasyprocta at Villavicencio for susceptibility to yellow fever virus using small doses of the Asibi and Martinez strains Of the animals from the region bounded by the Guacavia River on the north and the Guayuriba River on the south approximately 20 per cent gave initially positive protection tests None of these circulated virus after inoculation while the majority of those with pre experimental negative protection tests gave evidence of circulating virus for *variable periods between the 3d and 10th postinoculation days* The level of circulating virus varied from a trace to a magnitude of 10 [8] There were no signs of illness that could be attributed to the infection with yellow fever virus There was a moderate mortality incidental to the bleedings The postexperimental protection tests performed 30 days after inoculation were consistently positive in animals that had shown circulating virus an appreciable fraction of the remainder also developed antibodies

The laboratory evidence on the specificity of the antibodies developed *following the inoculations was satisfactory In addition* there was a strong presumption that most if not all of the positive protection test reactions with the first sera taken following capture were related to real immunity because virus failed to circulate following inoculation in initially positive animals These findings were of interest in light of the very high incidence of positive protection tests in animals that had been captured by Boshell Manrique near Restrepo (north of Villavicencio) in 1936–1937 following an extensive outbreak of yellow fever When Kerr tested 47 of these sera by the intraperitoneal procedure 17 gave results either frankly positive or classifiable as inconclusive However Bugher (1940) subsequently showed that the intraperitoneal test did not differentiate weak positives and that *the so-called inconclusive* results were actually indicative of considerable

virus neutralization He retested the remaining 30 negative sera in 1 110 after prolonged storage using the more sensitive intracerebral technique 10 of the 30 gave positive results bringing to 27 the total of positives in n the original 47 sera While it seems likely that the testing of the stored sera with the more sensitive intracerebral procedure gave valid results in i the 10 additional positives were also specific there is no information cerning the effect of storage in glass ampules upon such sera Theref would seem prudent to accept these interpretations with some caution pe l ing further study of the behavior of agouti sera after storage

The susceptibility of the Colombian agouti to the 17D strain of virus v demonstrated by Bevier, who made subcutaneous inoculations of yell v fever vaccine into four animals All circulated virus for several days be ginning with the 3d day and developed neutralizing antibodies

Twelve dasyprocta consisting of specimens of *Dasyprocta agouti mar ixi Dasyprocta azarae aurea* and *D agouti* were tested by Laemmert 1 15 using minute doses of virus Seven developed antibodies although circu lating virus was not demonstrated In some of the animals the demon tral l antibodies disappeared rapidly

In summary it would appear that in certain regions this rodent may take an active part in the epidemiology of the disease becoming infected a l circulating virus The virus titers are not high so that the problem of evalu ating the exact position of dasyprocta in particular cycles is similar to that presented by the marsupials

Hydrochoerus capybara

Largest and most powerful of all the rodents. Reaches a huge size adult male may weigh as much as 50 kg Appearance best described as resembling an enormous guinea pig Coat brown composed of rather coarse hairs Tail rudi mentary Possesses several adaptations for aquatic life particularly webbing of the feet and the ability to close the nostrils Herbivorous subsists on grasses and plant shoots growing in swamps and about pools Large incisor teeth can inflict serious wound

At Villavicencio Bugher Boshell Manrique and Gilmore made suscepti bility tests on 18 animals of this species With relatively small doses of the Martinez and Asibi strains most of them showed circulating virus in mod erate amounts for variable times between the 4th and 10th postinoculation days The titers were similar to those encountered with the agoutis and the pacas being in the neighborhood of 10 ² Postexperimental protection tests

at 30 days showed clearly developed antibody in all the animals that had shown circulating virus and also in most of those that had failed to circulate virus There were no signs of illness due to the presence of the virus, the deaths being incidental to the experimental manipulation

In addition to the animals captured alive, another dozen, chiefly old adults, were shot for specimens Positive protection tests, indicating previous infection in the wild state, were not obtained with sera from any of these animals The habits of capybara make it very unlikely that they ever play a significant part in the epidemiology of yellow fever, although from the standpoint of susceptibility they would appear capable of sustaining virus in cycles if they were in contact with a suitable vector.

Other *Rodentia*

There are some other rodents that appear to have some degree of susceptibility The common bush or spiny rat of the Colombian llanos, known as either *Proechimys chrysaeolus* or *Proechimys cayennensis o'connelli* (J. A Allen) was found by Bugher, Boshell Manrique, and Gilmore to be entirely refractory to virus inoculation in the laboratory It showed no circulating virus and exhibited antibodies only if massive doses of virus were used However, a clearly positive protection test was encountered occasionally in the wild population Since nonspecific reactions did not occur, these occasional positives were left without explanation until Laemmert (1948) found that among proechimys there is not only a marked species difference in susceptibility but also considerable difference in the response of a single species to the various strains of virus Using the Asibi, J Z, and O C strains, he found that *Proechimys dimidiatus* was susceptible to all three, *Proechimys iheringi* was susceptible only to Asibi, and *Proechimys cayennensis roberti* was resistant to all three

Laemmert also found that the Brazilian squirrel *Sciurus ingrami* circulated virus readily after inoculation with small doses of the Asibi strain and that subsequently there was a consistent development of neutralizing antibodies in all the animals tested. Tests by Bugher, Boshell Manrique, and Gilmore on a very small number of the corresponding squirrel of the Colombian llanos, *Sciurus igniventris*, revealed an apparent resistance to the Asibi strain

The wild guinea pig—*Cavia anolaima* in Colombia (Bugher, Boshell Manrique, and Gilmore) and *Cavia aperea* in Brazil (Laemmert, 1948)—

showed great variability in susceptibility. Some individuals circulated virus while others did not, apparently depending not only upon the virus strain used for testing but also upon the particular population sampled.

Among other rodents there is great variability in reaction pattern to yellow fever virus, as shown by Laemmert (1948), so that generalization is impossible. However, save for the species already mentioned, there would appear to be no instances of marked susceptibility.

EDENTATA

Within the order of edentates there are some apparently weird associations of superficially dissimilar animals. Even the name of the order is belied by the fact that some of the genera have very effective dentition. The edentates include the armadillos, the anteaters, and the sloths. Of especial interest are the armadillos, which are very numerous in some yellow fever areas.

Dasypus novemcinctus

Nine-banded armadillo. Very widely distributed in northern South America and very prevalent in the entire Guiana region. Taxonomically does not appear to vary greatly from one area to another. Markedly subterranean. Diet consists mainly of roots, grubs, and burrowing insects. Spends a great part of its time burrowing and away from the light, hence its contact with such mosquitoes as haemagogus is practically nil. Furthermore, its extensive armor makes it a difficult object of attack for any mosquito.

Whitman inoculated the common Brazilian armadillo with the Asibi strain of virus and found that it had appreciable circulating virus for several days. The same species in Colombia was tested by Bugher, Boshell-Manrique, and Gilmore with small inoculations of the Asibi and Martinez strains. Circulating virus appeared in moderate amount (titer approximately 10[illegible]) between the 3d and 7th postinoculation days and persisted for not more than 1 day. Thirty days following inoculation, antibodies were readily demonstrable by the intracerebral protection test.

Positive pre-experimental protection tests were encountered with considerable frequency under circumstances that seemed to preclude previous contact with yellow fever virus, and some animals circulated virus in the presence of such positive tests. It was concluded that an appreciable

number of nonspecific reactions took place with the technique in use which involved storing the sera for a month in the Wassermann tubes employed for the protection tests Since there were only the single pre experimental bleedings it was impossible to determine the origin of the nonspecific results However Whitman who studied the same species in Brazil never encountered any positive reactions using fresh sera and a sensitive protection test Therefore it seems highly likely that some of the results at Villavicencio were related to the manipulation and storage of sera and not to the animals themselves This was reflected in the A S T values and the variance of these in the proved nonimmune animals Compared to the primates both the mean A S T and the standard deviation were appreciably increased Recent comprehensive tests by Hughes and Perlowagora (1950b) on a number of South American mammals including armadillos extended and confirmed earlier observations on these edentates

Circulating virus and effective antibody production were found in the few *Dasypus kappleri* tested This species did not appear to differ significantly in behavior from its relative *D novemcinctus*

Tamandua tetradactyla (L)
Myrmecophaga tridactyla (L)

The anteaters of South America fall into three groups of which *T tetradactyla* (medium sized anteater) and *M tridactyla* (giant anteater) represent two (The third is the very small arboreal anteater *Cyclopes didactylus* of which no representatives have been tested with yellow fever virus) Anteaters are highly specialized and admirably adapted to their diet and mode of life Possess powerful forelegs armed with tremendous hooklike claws for tearing open anthills or termite nests with ease Have long tongue with sticky surface for gathering up insects and carrying them into the mouth Quite difficult to maintain in captivity but can subsist for a time on a diet of milk and raw eggs

T tetradactyla was first tested for susceptibility to yellow fever virus by Whitman who used subcutaneous inoculations of Asibi virus The animals showed circulating virus from the 3d day to the 7th and within a month well defined antibodies were demonstrable by the intraperitoneal protection test There was no evident illness as a result of virus multiplication although the usual difficulty was encountered in keeping the experimental animals alive

The same species occurs immediately east of the Andes at Villavicencio

and was tested by Bugher, Boshell-Manrique, and Gilmore with essentially similar results. An occasional animal was found to have a naturally positive protection test; in the absence of any evidence of nonspecific reaction, these naturally occurring positive reactions were considered to be indicative of previous infection with the virus.

Only two specimens of *M. tridactyla* were available for experimentation. The results were closely parallel to those obtained with the smaller ant-eater.

Both of these species are accustomed to move about slowly during the day through the marginal portions of forests, thus favoring mosquito attack and engorgement. Against their playing any major part in the epidemiology of yellow fever is their low population density. No region has been reported where the animals constitute any appreciable fraction of the mammalian population susceptible to the virus of yellow fever.

Choloepus didactylus

Sloth. Comparatively less common than either of the two species of anteater discussed above; usually encountered during the season of felling trees for new farm land. Slow and chiefly nocturnal. Coat reddish-brown, long and shaggy. Each foot armed with one large and one small claw, which aid in moving about in the trees and in digging in tree bark and termite nests for insects and grubs which form part of the sloth's diet.

Only one sloth was available for experimentation by Bugher, Boshell-Manrique, and Gilmore at Villavicencio. This animal had a negative pre-experimental protection test and circulated a small amount of virus following subcutaneous inoculation of a small dose of Asibi virus. The postexperimental protection test was positive. The undiluted serum was highly toxic to mice on intracerebral inoculation, as has been noted with many wild animals. Although the indications were that the animal is susceptible to yellow fever virus, it was not considered necessary to extend the studies because the scarcity of the animals alone would exclude them from any significant participation in the forest cycle of yellow fever.

CARNIVORA

In general, the entire order of carnivores is resistant to yellow fever virus of any strain, but there are certain exceptions, the most important being

Potos flavus or the kinkajou This small and attractive animal is readily infected and after a period of virus circulation lasting several days often with titers between 10^{-2} and 10^{-3} produces well defined antibodies and is effectively immune The occasional wild caught specimen with a positive protection test is apparently specifically immune since such kinkajous do not circulate virus when inoculated *P flavus* is another primarily nocturnal animal that may be encountered also during daylight hours It usually sleeps in dark tree holes which tend to protect it from haemagogus mosquitoes but it also may be found sleeping in the canopy on the limbs of trees where it is perfectly accessible to the mosquito vectors

UNGULATA

Tagassu tajacu
Tagassu pecari

White collared and white-lipped peccaries form a large population of vast extent in northern South America as well as in Central America and Mexico In the Orinoco basin are found the two main types *T tajacu* and *T pecari* Tajacu in Colombia occur in small bands of about a dozen animals Tajacu considerably smaller than *T pecari* Comparatively timid when alarmed take flight sometimes going to ground in burrows In contrast pecari tend to occur in large bands sometimes up to several hundred individuals roaming widely and aggressively When alarmed these animals often attack rather than flee and rarely take refuge in caves or burrows In large herds white lipped peccaries can be dangerous to humans

Only *T tajacu* the white-collared species was tested at Villavicencio by Bugher Boshell Manrique and Gilmore Upon inoculation with the Asibi strain these animals circulated virus in small amounts and developed neutralizing antibodies as shown by the postexperimental protection tests made 30 days or more after the inoculation There was no recognizable illness due to the infection The serum of the normal animals was found to have a definite virus inactivating character so that the protection tests on non immune animals would be interpreted as positive if the standards for the rhesus monkey were used This nonspecific activity was not present in the dilutions of the normal serum whereas antibody could be readily shown in dilutions of the serum from the actively immunized animals

Among the comparatively few animals shot for survey purposes an occasional positive reaction was obtained with specimens from known yellow

fever areas the interpretation allowing for the nonspecific property of the serum itself It may be that the peccaries play a in the jungle yellow fever picture of limited areas but at p are not sufficient data to sustain such an hypothesis On the othe certain that in many regions where yellow fever is endemic t are either absent or very scarce

Sus scrofa domestica

Domestic pig Following the demonstration of the susceptil peccary to the virus of yellow fever Bugher Boshell Manrique tested six young domestic pigs in a similar manner Four anii circulating virus following inoculation of a small dose of Asibi animals also produced well defined antibodies As with the pe mal pig serum *proved to be definitely virucidal so that the pro* were conducted with a greater virus dose than in the case of t When tested with due regard for the nonspecific virus inactivat of the normal serum there was no difficulty in demonstrating ment of antibodies

In South America pigs are commonly kept in small num farms that are in the jungle yellow fever areas It is possible t the pigs like the peccaries may make some contribution to th yellow fever virus It seems highly unlikely however in view tively small numbers of such animals in any endemic area that pig ever plays a significant part in the basic epidemiology of th

Other *Ungulata*

Not only pigs but also cattle and sheep may exhibit nonspe protection tests Findlay Stefanopoulo et al (1936) establishe in England would in some instances give strongly positive test quently protection tests of cows in yellow fever areas had no v Sabana de Bogotá Colombia where the altitude of nearly 9 *ensured freedom from yellow fever* *It was found that* approxi third of 50 cows gave positive protection tests However such a could be used in 10 per cent concentration in tissue cultures virus without creating any difficulty whereas specifically imm serum could not be employed in a concentration greater than The occurrence of such nonspecific immunologic reactions d clude susceptibility on the part of the animal the two phenom

be entirely unrelated However, none of the ungulates except those already discussed have been shown to be susceptible so far although the studies of many of them have been very meager

AVES

A great amount of work has been expended on the question of the susceptibility of birds especially in Brazil where the subject has been most investigated Various virus strains have been used and tests for circulating virus and antibody production have been made following inoculation The results may be summarized as entirely negative

The chief information of positive character that has resulted has been that with certain species such as buzzards nonspecific positive protection test reactions are very frequent Consequently the same problem exists in the birds as in many other animals namely that protection test results cannot be interpreted without carefully controlled studies on the behavior of each species with respect to the virus

At Villavicencio considerable direct experimentation was done by Bugher Boshell Manrique and Gilmore on buzzards pigeons and parakeets using the same general pattern as was employed for the testing of mammals In every case the original inoculum was rapidly cleared from the circulating blood and virus did not subsequently reappear in the circulation With pigeons the rate of virus clearance was affected by the temperature of the bird clearance was slowed up appreciably when the pigeon was chilled to a catatonic state

Small virus inoculations produced no change in the protection test results but after inoculation of large doses of virus a moderate antibody response could be demonstrated A considerable frequency of nonspecific reactions was observed especially among the buzzards which consistently gave positive protection tests even with fresh sera and with other species notably pigeons when the sera were stored for a few months These events were not predictable and appeared to result from a number of factors some of which may have been related to the techniques employed for preparing the sera It was clearly evident that protection tests among birds require a great amount of detailed serologic study of each species before they can be interpreted at all As far as the evidence now available indicates a positive protection test in a serum specimen from any bird should be considered

nonspecific until proved otherwise by further studies of the species behavior This is equivalent to stating that the burden of proof is reversed from that in other orders, such as the primates

POIKILOTHERMIC ANIMALS

Considerable numbers of the cold blooded animals were tested at Villavicencio by Bugher Boshell Manrique and Gilmore Among these were various species of frogs toads lizards snakes and turtles In no instance was



FIG 44 Dr John C Bugher working at a field laboratory table while on a yellow fever expedition in the llanos of Colombia (1940)

there any evidence of multiplication of virus and antibody could be excited only by the massive inoculation of virus

Studies initiated at Villavicencio by Laemmert (1918) were continued in Brazil with considerable extension of the coverage of the species tested, but with uniformly negative results

These results were foreshadowed by the earlier investigations of Sawyer (1931*b*) on the survival of yellow fever virus in frogs and toads kept in the cold There was good survival but no evidence of any multiplication of the virus

THE AFRICAN VERTEBRATES

The susceptibility of the African fauna to yellow fever virus has received far less study than that of the South American fauna Although there is a broad base of jungle yellow fever in Africa the overwhelming majority of human cases are due to the man—*A aegypti*—man cycle The occasional acquisition of the virus by man from forest mosquitoes tends to be obscured by the mass of cases resulting from the classic epidemiologic cycle In South America it was the occurrence of yellow fever in areas free of aegypti either naturally or as a result of antimosquito campaigns that threw into sharp relief the importance of endemic yellow fever among the animal populations Not until the work in South America had revealed the existence of the disease among certain of the animal species was it realized that a comparable situation exists in Africa The demonstration of the susceptibility of the rhesus monkey to yellow fever and the isolation of the Asibi strain of virus (Stokes Bauer and Hudson 1928*a* and *b*) naturally stimulated further interest in the wild monkeys these events also reactivated the part of the program of the Yellow Fever Commission to the West Coast of Africa that was to have included studies of the monkeys (Fowler, 1931)

Since the primates constitute an enormous population in Africa and are so clearly fundamental in the yellow fever picture they have received most of the attention time has not yet permitted any detailed studies of other mammalian orders Another factor that contributed to the emphasis given the primates in Africa is that in Africa jungle yellow fever has not been observed except in the presence of monkeys No situation analogous to that of the Muzo region of Colombia where endemic yellow fever exists in the absence of monkeys has yet been found in Africa The discussions of the susceptibility of African animals are thus very incomplete and a vast amount of work remains to be done before a comprehensive picture can be given

PRIMATES

The nomenclature of the African primates an inherently complicated matter has been rendered almost chaotic by the differing preferences of taxonomic authorities In the main G M Allen (1939) has been followed here since his work represents the totality of information available on the

subject up to 1938 however considerable descriptive material has been obtained from Elliot (1913)

Systematic presentation of all the species is impossible in a discussion of this scope Attention has therefore been given here only to primates known to be of importance with regard to the yellow fever problem of the areas studied The general outline of the genera considered is as follows

Suborder LEMUROIDEA (lemurs)
Family *Lorisidae* (galagos and slow lemurs)

Subfamilies

A *Lorisinae*

Genera 1 *Arctocebus* (golden potto)

2 *Perodicticus* (pottos)

B *Galaginae* (galagos or bush babies)

Genera 1 *Galago* (29 species described)

2 *Euoticus* (2 species)

Suborder ANTHROPOIDEA (monkeys and apes)
Family *Cercopithecidae*

Genera 1 *Allenopithecus*

2 *Cercocebus* (mangabeys) (10 species)

3 *Cercopithecus* (guenons)

C aethiops group (18 species)

C cephus group (2 species)

C diana group (3 species)

C l hoesti group (3 species)

C mitis group (20 species)

C mona group (10 species)

C neglectus group (1 species)

C nictitans group (12 species)

C talapoin group (3 species)

4 *Erythrocebus* (red monkeys) (3 species)

5 *Comopithecus* (hamadryas baboons) (2 species)

6 *Macaca*

M sylvanus (L) (barbary ape)

7 *Mandrillus* (mandrills) (9 species)

8 *Papio* (baboons) (16 species)

Family *Colobidae* (leaf eating monkeys)

Genus *Colobus* (colobus monkeys)

C. polykomos group (19 species)

C. badius group (20 species)

Family *Pongidae* (great apes)

Genera 1. *Pan* (chimpanzees) (4 species)

2. *Gorilla* (gorillas) (2 species)

Pan troglodytes troglodytes (Fig. 43)

Chimpanzee. The distribution of the chimpanzee in Africa is discontinuous. Considerable numbers occur in Sierra Leone and adjoining French Guinea. Eastward along the coast they are very infrequent until the British Cameroons are reached. They occur continuously through the Cameroons, French Equatorial Africa and the Belgian Congo to East Africa, especially in western Uganda. But nowhere do they constitute a dense population and they are always few in number in comparison with the monkeys. It is curious that the susceptibility of an animal other than man to the virus of yellow fever was first demonstrated in an African primate not in its native habitat but far up the Amazon River at Manaus, Brazil. Thomas (1907) of the University of Liverpool expedition to Manaus obtained a chimpanzee from Sierra Leone. In November 1906 he exposed the animal to the bites of aegypti that had fed 21 days previously on two patients ill with fatal yellow fever. Within 3 days the animal had fever and was obviously ill, but there was no vomiting or observable icterus. There was gross bilirubinuria on the 10th and 11th postinfection days, diminishing thereafter, which indicated that the liver had suffered some damage despite the absence of icterus earlier. Granular casts and some red blood cells were present in the urine from the 4th to the 10th postinfection days. The fever began to drop on the 10th postinfection day and was normal by the 14th. The chimpanzee recovered gradually, and thereafter it could not be reinfected by the bites of virus carrying mosquitoes.

Other studies were made and it is likely that some of the local monkeys were shown to be susceptible by the same technique (Franco, Martinez Santamaria and Toro Villa, 1911). Unfortunately Thomas died early and after his death a laboratory fire destroyed his records, so that much of the very significant work at Manaus was lost. However, informal communica

The results however have been strikingly concordant and together form a pattern that probably also holds true for the intervening forests of the Belgian Congo and French Equatorial Africa although a vast amount of work still remains to be done before this statement can be accepted as more than a probability

The isolation of the virus of yellow fever by Bauer and Mahaffy at Accra Gold Coast and the further studies of this virus (which came to be known as the Asibi strain) at Lagos by Stokes Bauer and Hudson (1928*b*) gave great impetus to the clarification of the epidemiology of yellow fever in man Extensive use of the rhesus monkey as an experimental animal made possible the acquisition of exact knowledge about the character of the virus in addition by means of the rhesus protection test the distribution of immunity in the human population could be demonstrated Meanwhile workers at the Pasteur Institute at Dakar French West Africa were meeting with similar success The virus strains isolated there by Sellards and Laigret (1928) were further studied by Hindle (1929) who not only confirmed Hudson s (1928) work in pathology but also called attention to the difference between the virulence of the virus for the rhesus monkey and its virulence for man

Using the French strain of virus Pettit Stefanopoulo and Kolochine (1928) found no evidence of reaction in two *Cercopithecus griseo viridis* two *Cynocephalus hamadryas* and one *Cynocephalus papio* Pettit and Stefanopoulo (1929) conducted further experiments with two *C griseo viridis* two *Cercopithecus callitrichus* two *Papio sphinx* and four *C hamadryas* and again obtained no signs of infection Still later Pettit and Stefanopoulo (1930) found that *Macaca inuus* (= *Macaca sylvanus*) from North Africa reacted like the rhesus They concluded that all of the African monkeys (save the North African macaca) were refractory to the virus

These and previous failures to demonstrate the susceptibility of the West African monkeys to yellow fever were then reviewed in the light of the known behavior of the virus in the rhesus Bauer and Mahaffy (1930*a*) obtained specimens of the following species the first two of which are the most common monkeys in southern Nigeria *Cercopithecus tantalus* (Ogilby) *Cercopithecus mona* (Schreber) *Cercocebus torquatus* (Kerr) (Fig 45) and *Erythrocebus patas* (Schreber) All were given virus either by direct inoculation or by the bite of aegypti mosquitoes infected on a rhesus monkey ill with yellow fever By subinoculating the blood of the experimental animals into rhesus monkeys Bauer and Mahaffy demonstrated that al



Fig 45 *Cercocebus torquatus torquatus* (white collared or red headed mangabey) (*Courtesy of New York Zoological Society*)



Fig 46 *Colobus polykomos ruwenzorii* (mountain colobus)



Fig 47 *Perodicticus potto potto* (Bosman's potto)



Fig 48 *Galago demidovii demidovii* and twin young

though none of these African monkeys showed significant signs of illness the virus multiplied greatly and circulated in their blood for several days Further mosquitoes fed on *C tantalus* and *C torquatus* were later able to transmit the virus by bite to rhesus monkeys The seven monkeys that survived the experiments were bled 3 weeks or more after infection and the sera were examined for antibodies by the rhesus monkey protection test all seven had clearly demonstrable antibodies All of the five available pre experimental sera were negative in the rhesus protection test The postexperimental reactions actually indicated solid immunity since second inoculations of virus did not provoke the appearance of circulating virus Bauer and Mahaffy concluded that the monkeys were susceptible to the yellow fever virus even though they did not manifest clinical signs of disease and that they could be infected by the local mosquitoes including species other than *A aegypti* some of which live in the forest

The fundamental importance of these experiments was not realized until several years later when it began to appear that the classic man–aegypti–man cycle is only a special phase of a much broader epidemiologic pattern in which animals play the basic role Although the following years brought improved and simpler virus techniques these earlier experiments established the essential character of monkey yellow fever The subsequent much more extended investigations have confirmed the findings of Bauer and Mahaffy without any additions of fundamentally different nature

Later Theiler and Hughes (1935) obtained in New York City some specimens of *Lasiopyga callitrichus,* a species related to Bauer and Mahaffy s *C tantalus* and tested these animals with the Asibi strain using white mice to demonstrate the presence of virus They too found that the animals showed no illness but did circulate virus and that the duration of virus circulation was inversely related to the size of the infecting dose This pattern was shown to be like that observed in the rhesus monkey when variable infecting dosages were used Antibody production in *L callitrichus* was demonstrable with ease by the mouse protection test and Theiler and Hughes concluded that the antibody response was the same in both so called susceptible and relatively insusceptible monkeys

At Entebbe Uganda it was necessary to put the major emphasis on demonstrating the existence of the human disease and the presence of the virus so that studies in monkeys had to be discontinued for a time Later they were resumed Hughes (1943) investigated the susceptibility of the common gray monkey *Cercopithecus aethiops centralis* using Asibi virus both by inocu

lation and by bite of infected *A aegypti* and *Aedes metallicus* mosquitoes Daily bleedings were made and the virus levels were determined by titration of the sera in mice The circulating virus magnitudes are shown in Table 13 As can be seen, there was considerable variability in the time during which virus circulated and the amount of virus present in the blood stream In general the animals exhibited no outward signs of illness due to the virus except for one young monkey whose liver after death showed lesions similar to those found in the rhesus The other deaths were from incidental causes The lack of visible illness combined with the circulation of considerable amounts of virus indicated a pattern of infection such as had been demonstrated by Bauer and Mahaffy (1930*a*) in West Africa

TABLE 13

SUSCEPTIBILITY OF C AETHIOPS CENTRALIS TO YELLOW FEVER VIRUS

Animal no	*Virus strain*	*Circulating virus as log LD_{50}/cc*									
		Days postinoculation									
		1	2	3	4	5	6	7	8	9	10
12	Asibi Inoculation		2 15	5 77	6 52	6 52	6 52	6 52	dead		
13	Asibi Inoculation		3 19	6 02	6 52	6 52	4 37	3 02	1 75	dead	
14	Asibi Inoculation		1 82	4 28	6 83	2 02	1 93				
15	Asibi Inoculation		2 17	7 02	3 92	1 98	dead				
16	Asibi *A metallicus* (4)			4 02	4 36	5 02	0	0	0	0	0
17	Asibi *A aegypti*	0		3 89	6 02	3 89					
18	Asibi *A aegypti*		3 22	6 02	6 08	2 06	1 52				
19	Asibi *A aegypti*		2 39	0	5 26	1 52					
20	Asibi *A aegypti*						3 17	3 22	2 33		
21	Asibi *A aegypti*	0	0	0	5 83	1 52					
22	Asibi *A aegypti*	0	4 52	6 52	7 12	8 52	6 89	dead			

The East African monkeys baboons, and apes were further examined by Smithburn and Haddow (1949) in a very extensive coverage of the species encountered in Uganda The majority of the animals in this series showed a moderate rise in temperature The highest temperature recorded was 105 6°F, but most of the maxima were between 103 and 105°F These were

TABLE 14 (*Part I*)

EAST AFRICAN PRIMATES

SUSCEPTIBILITY TESTS AND ANTIBODY PRODUCTION

An mal no	Spe es	Virus strain dosage as mouse LD_{50}	Circulat ng virus as log LD_{50}/cc								Protection tests serum first protective day
			Days post inoculat on								
			3	4	5	6	7	8	9	10	
45	*Cercocebus alb ena johnstoni*	As 1 92				1 43	0 50				10
278	*Cercop thecus aeth ops central s*	Lg As 42 20	4 12	6 52	7 78	6 78	5 00	4 00			8 died 9th day
2 4	*C l hoesti l hoe ti*	Lg As 42 20			1 83	2 52					9
486	*C l hoesti l hoesti*	As b $10^{2.52}$	6 21	6 36	1 77						6 *
566	*C m t s k bondensis*	As b 460	4 12	4 89	1 83						6
567	*C m t s kibonolensis*	As b 460	3 89	5 89	1 52						6
52	*C m t s k bonolensis*	As b 92	3 89	6 28	5 02	2 89	1 65				7 died 16th day
752	*C mit s kolb*	As bi 92	4 89	7 77	6 15	0 50	0 50				8
754	*C mitis kolbi*	Lg As 44 24	1 52	2 36	5 05	3 92	0 50				8
26	*C m tis stuhlmann*	Ug As 42 92	4 28	5 77	4 92						10 *
859	*C m t s stuhlmanni*	Ug As 44 120	3 77	4 89	4 92						7
277	*C n ct tans mpangae*	Lg As 42 20		1 52	3 52	4 39	5 02	4 02	2 92		Neg 9th 1nd 10th day
491	*C n t tans mpangae*	As b $10^{2.52}$	2 22	3 39	0 50						6 *
235	*Erythrocebus patas pyrrhonotus*	Lg As 42 20	2 00	4 12	5 92	5 02					Incon lus ve 7th died 8th day
564	*Eryth ocebus patas pyrrhonotus*	As bi 460	2 92	3 13	4 52	2 22	1 52				8
265	*Pap o doguera tessellatu*	Lg As 42 92	2 88	4 52	4 36	0 50					10 *
266	*Pap o doguera tessellatu*	Lg As 42 92	2 88	5 77	3 39						10 *
540	*Colobus polykomos k kuyuen s*	As b 269	3 67	7 52	7 82	6 52	5 52	4 77	4 02	1 27	12 * died 12th day
549	*Colobus polykomos k kuyuensis*	Asib mosquito	0 50	4 52	5 12	3 88	2 16	1 52			8
711	*Colobus polykomos uellen s*	Asibi 6	2 92	3 67	6 36	6 52	5 16				Died 8th day
908	*Pan troglodytes schweinfurth i*	Ug As 44 400	3 52	3 16	2 37						8

* Not tested previously

ificant elevations above the normal for these animals and were there interpreted as true fevers The ambient temperature at Entebbe is con rably lower than it is at Lagos, which may account for the lower mean nal temperature of experimental monkeys

aily blood specimens were examined quantitatively for circulating virus, the residual sera were tested for antibodies (Table 14 Part I) The table ws the first day on which antibodies were definitely demonstrable Actu , in nearly all of the animals, earlier blood specimens showed the pro ytion of the A S T indicative of the presence of antibody in small ount Part II of Table 14 gives the results of massive doses of virus Here ulating virus appeared very early in fact was continuously present fol ing the inoculation, reaching an early maximum and then quickly fall to zero This is a generally encountered phenomenon where large inocu ons of virus are used

TABLF 14 (*Part II*)

FAST AFRICAN PRIMATES

SUSCEPTIBILITY TESTS AND ANTIBODY PRODUCTION

Spe ies	Virus strain dosage as mou e LD_{50}	Circulating virus as log LD_{50}/cc										Protection tests serum first protective day
		Days postinoculat on										
		1	2	3	4	5	6	7	8	9	10	
Cercopithecus aethiops centralis	Ug As 42 $10^{7\ 21}$	>1 52	2 01	2 92	1 40							15 *
C aeth ops central s	As b $10^{7\ 46}$	3 52	4 11	1 00								10 *
C mitis stuhlmanni	Ug As 42 $10^{7\ 2}$	1 30	4 23	t ace								15 *
C nict tans mpangae	Ug As 42 $10^{7\ 16}$	>1 52	3 02									15 *
Papio deguera tessellatus	Ug As 42 $10^{7\ 21}$	>1 52	4 02	1 77								15 *

arl est bleed ng tested

With a few exceptions all the animals used in these experiments developed culating virus in amounts adequate to infect a significant proportion of y vector species of mosquito that might feed on them An occasional ani il showed a low virus titer throughout the experiment, but this appeared have no correlation with any particular species and may be considered as rt of the inherent variation to be expected It appears safe to generalize d state that all of the East African monkeys studied are capable of infect

ing susceptible forest mosquitoes with considerable regularity and can play an active part in the general forest cycle This conclusion is supported by the serologic studies on the monkey population of Bwamba County carried out by Haddow Smithburn et al (1947)

Another finding of considerable importance is the frequency with which demonstrable antibodies may occur in the sera of monkeys at a time when virus is still circulating As brought out in other chapters of this volume the simultaneous occurrence of virus and antibody may significantly affect not only the ease of serial passage in animal passage by direct inoculation but also the establishment of the virus in susceptible mosquitoes

Seven species of West African monkeys and baboons were tested by Bugher and Hahn with a small dose (540 L D_{50}) of the Ojo strain of yellow fever virus isolated in 1946 by Bugher Hahn et al at Ogbomosho Nigeria Bleedings were made from the 3d to the 10th days and the virus levels determined by titration in mice In addition bleedings were made at 10 and 30 days and the sera tested for antibodies in two types of protection tests subcutaneously in baby mice (3 day) and intracerebrally in adult mice the same serum virus mixtures being used for the two tests The animals all had clearly negative protection tests before being inoculated The results of the experiments are summarized in Table 15

Again there is to be noted the marked variability with respect to the time characteristics and the quantity of circulating virus All of the animals tested apparently had enough virus at some time to infect mosquitoes Of considerable interest was the finding that the antibody response had some distinctive characteristics similar to those encountered by Bugher in human sera Early in convalescence many human patients form antibodies which are readily demonstrable by the subcutaneous protection test in baby mice but which are detected only with difficulty by the intracerebral test in adult mice With time the intracerebrally effective antibody steadily rises so that after 2 or 3 months the results of the two tests may not differ greatly The comparative behavior of the two tests can be evaluated by noting the results of control titrations of pooled human immune sera from persons immunized to yellow fever several months to several years previously The titers with the immune pools are typical and show that the subcutaneous baby mouse test is inherently somewhat more sensitive than the intracerebral adult mouse test but that the difference is a minor one It is significant that the full development of neutralizing antibodies demonstrable in the intracerebral test may require several weeks

TABLE 15
West African Primates
Susceptibility Tests and Antibody Production

Animal no	Species	Dosage of Ojo strain of virus	Circulating virus, as log LD_{50}/cc								Protection tests, antibody titer			
			Days postinoculation								10 days p i		30 days p i	
			3	4	5	6	7	8	9	10	s c	i c	s c	i c
											1	1	1	1
A 17	*Cercopithecus mona mona*	540 LD_{50}			3 26	4 65	5 77	3 92	2 82	2 36	Neg	Neg	1 000	24
A 433	*C mona mona*	540 LD_{50}	3 77	4 12	3 91	1 52	1 52				49	6	480	21
A-388	*C aethiops tantalus*	540 LD_{50}	4 17	5 17	4 23						480	10	250	30
A 434	*C aethiops tantalus*	540 LD_{50}	3 52	4 12	3 92	1 52	1 52				10	5	320	77
A 21	*Cercocebus torquatus atys*	540 LD_{50}	2 78	5 18	4 12	1 52					1 000	2	480	166
A-439	*Cercopithecus nictitans erythrogaster*	540 LD_{50}	2 92	3 37	4 36	1 52					3	3	480	8
A 431	*Erythrocebus patas patas*	540 LD_{50}	1 52	2 85	4 17	3 88	4 18	2 37	1 52	1 52	Neg	Neg	140	11
A 429	*Papio nigeriae*	540 LD_{50}	3 27	3 80	2 64						1	2	320	37
A 430	*P nigeriae*	540 LD_{50}	3 18	3 03	3 52						6	1	100	34
A 438	*Mandrillus leukophaeus*	540 LD_{50}	2 90	3 27	6 18	3 90	3 27	2 13	1 52		4	20	320	21
	Controls													
	IHSP—6										45	10		
	IHSP—5												90	60

Note p i = postinoculation
s c = subcutaneous
i c = intracerebral
IHSP = immune human serum pool

While these experiments give no information on the duration of the demonstrable immunity there is indirect evidence to show that in the great majority of monkeys demonstrable antibodies persist for life In an endemic yellow fever area one rarely finds an old adult with a negative protection test indicating that loss of antibodies if it occurs at all must be infrequent There is no evidence that the African monkeys behave at all differently in this respect from man

In the series of Smithburn and Haddow one *C aethiops centralis* and one *Colobus polykomos* (Fig 46) died from yellow fever alone yellow fever either bore no relation to the other deaths or constituted only a minor contributory factor Some of the animals showed significant fever although Bugher and Hahn encountered no febrile or clinical reactions in their series It appears therefore that severe illness ending in death is comparatively infrequent among the species considered in this section of the discussion and that most of the individuals fail to show any outward sign of the infection Such manifestations as do occur are unrelated to the amount of circulating virus hence the subclinical infections are just as significant epidemiologically as the most severe ones

Lemuroidea

The lemurs are distinctly different from other primates in their behavior with respect to yellow fever virus and have received a considerable amount of detailed study The suborder includes the pottos and galagos both of which are comparatively numerous throughout central Africa They are all nocturnal and are encountered infrequently by day although they do move about some especially if disturbed The pottos and galagos are all arboreal and primarily insectivorous However the pottos in particular will avail themselves of whatever fruits and birds eggs may be at hand since their habit of moving slowly makes inanimate food more suitable to them In contrast all of the galagos are highly active executing magnificent leaps from branch to branch in the dark with the greatest accuracy With care galagos can be maintained in captivity over prolonged periods with very little loss

There are distinct habitat preferences among the various species of *Lemuroidea* especially among the galagos M Taylor in an extensive investigation of the galagos of Nigeria and the Cameroons obtained *G demidovii demidovii* in considerable numbers from the modified rain forest of Ilaro and Olokomeji Farther north in the zone where the rain forest begins to

merge into the savannah *Galago senegalensis senegalensis* was abundant and very closely associated with a single species of tree *Isoberlinia doka* The association appeared to result from two factors (*a*) *I doka* is the dominant tree species and (*b*) this tree is especially prone to the formation of deep holes where portions of the trunk rot away The galago was found to spend the daytime hours sleeping deep in such tree holes

To the east in the heavy rain forests of the Cameroons is found *Euoticus elegantulus elegantulus* a close relative of *G senegalensis senegalensis* This species was encountered in the high forest near Kumba and in the mountain forest between Mamfe and Bamenda *Galago crassicaudatus lasiotis* studied by Smithburn (1949) was obtained from the Gede and Kilifi coastal forests of Kenya near Mombasa while *Perodicticus potto ibeanus* (Thomas) was captured in Uganda

Perodicticus potto potto (Fig 47)

The general procedure for testing these animals has been as follows Small doses of African virus strains were used and the pottos were then bled daily The sera were subinoculated into mice in order to demonstrate the presence of circulating virus and were titrated in mice to determine the quantity of virus present Mouse protection tests were performed on the pre experimental and postexperimental sera in order to investigate the anti body production

In this manner Bugher and Hahn tested 16 *P potto potto* with the Asibi and Ojo strains of virus both from West Africa Nine animals showed multi plication of virus which in some individuals reached moderate concentra tions Virus circulated for periods varying between 1 and 6 days but the duration of circulating virus did not appear to be correlated with any other manifestations of the infection None of the animals were visibly ill and there were no deaths attributable to the virus

Antibody production was consistent and was readily demonstrated by the intracerebral protection test in mice False reactions were not encountered and the animals with positive protection tests following virus infection re mained positive for at least 6 months Studies on the long time persistence of demonstrable immunity have not been done

A few positive protection tests in captured animals have been encountered and may be taken as reliable indication that pottos are occasionally infected in nature It would seem probable however that the frequency of infection of the pottos is appreciably less than among the monkeys of the same regions

Smithburn (1949) tested three *P. potto ibeanus* with yellow fever virus originating in Uganda. All three showed circulating virus in moderate amount, and on the 10th day the sera of two were protective; the potto with the negative reaction was found to have been still circulating virus at the time. It was concluded that these animals also would be able to infect the forest mosquito vector.

Galago demidovii demidovii (Fig. 48)

This animal, the smallest of the primates, was studied by Bugher and Hahn for its response to the Asibi and Ojo strains of yellow fever virus. A considerable number of them was available as the result of the work of M. Taylor on the ecology of galagos.

In all, 52 animals received inoculations of Asibi virus; 13 showed circulating virus. The dosage of virus varied from 750 to 31,300 LD$_{50}$, and there was no correlation between the size of the infecting dose and the subsequent behavior of the animal. Among the 13 individuals showing circulating virus were seven with virus on a single day (either the 3d or the 4th), four showing it on 2 days, and two on 3 days. Only three circulated virus as late as the 5th postinoculation day.

The serologic studies indicated that the occurrence of circulating virus had no bearing on whether the animal subsequently gave a positive protection test, since approximately 50 per cent of both groups ultimately had neutralizing antibodies. Further, several of the animals developed positive protection tests only after an unusually long time. Most of the neutralizations were comparatively weak, although readily reproducible on retesting. Among the animals tested twice there was no instance of a positive at 1 month becoming negative at 3 months, although six individuals showed the reverse change.

It is felt that the findings with this species cannot be fully interpreted at the present time and more study is necessary. Possibly, the virus circulating on the 3d day was residual from the inoculation and did not in fact signify infection and multiplication of the infectious agent. The limitation of the demonstration to the early days of the postinoculation period, the small amounts of virus found, and the occurrence of virus on the 3d day in 10 of 13 experimental animals would all tend to support this hypothesis. However, further study is required before the implication of a primate genuinely refractory to yellow fever virus can be accepted.

TABLE 16

SUSCEPTIBILITY OF GALAGO SENEGALENSIS TO YELLOW FEVER VIRUS AND ANTIBOD

Animal no	Circulating virus as log LD_{50}/cc after inoculation of 100 LD_{50} ... Day 3	4	5	6	7	8	9	10	Protection test antibody: pre exp sc	pre exp ic	0 day post inoc 1	0 day post inoc 1	1
468	3 03	x	3 12	x	1 52	x		x	Neg	Neg			1
469	4 78	x	2 22	x	1 52	x		x	Neg	Neg			10
470	2 00	x	1 82	x		x	52	x	Neg	Neg			3
471	5 36	x	1 52	x	2 22	x	1 52	x	Neg	Neg			20
473	4 08	x	2 30	x	2 22	x	1 52	x	Neg	Neg			6
474	x	5 98	x	1 82	x	2 2	x		Neg	Neg	100	6	2 000
475	x	6 86	x	2 52	x	2 2	x	1 52	Neg	Neg	0	1	40
476	x	4 03	x	1 30	x	1 52	x		Neg	Neg	32	5	140
477	x	2 94	x	1 52	x		x		Neg	Neg	240	4	96
478	x	6 52	x	1 82	x	1 52	x		Neg	Neg	140	6	20x
Control human immune serum pool											16	74	66

Note sc = subcutaneous
ic = intracerebral

Galago senegalensis senegalensis

The ecology of this species which is much larger than *G demidovii demidovii* was studied by M Taylor its susceptibility to yellow fever was investigated by Bugher and Taylor As with *G demidovii demidovii* small doses of African strains of virus were used and the development of virus and antibodies was followed (Table 16) The behavior was closely parallel to that of the West African monkeys there being abundant virus multiplication but no illness Antibody production was effective and entirely consistent with the virus experience All animals tested showed circulating virus although as usual the amounts and the duration of the circulation were variable Antibody production was assessed by both the subcutaneous test in baby mice and the intracerebral test in adult mice the results were similar to those obtained with the monkeys In a series of over 50 animals captured near Zaria there was not a single pre experimental positive protection test It was concluded that while *G senegalensis* is fully susceptible to yellow fever the particular population examined had not participated in a forest cycle of the disease The country where this galago is found it will be recalled is not covered by true forest but instead by an open savannah forest with canopy

Galago crassicaudatus lasiotis

This species which was studied by Smithburn (1949) in East Africa is the largest of the galagos and comes from the coastal region of Kenya Using Asibi virus Smithburn obtained the circulating virus levels shown in Table 17 In contrast to *G senegalensis,* crassicaudatus had a high proportion of illness and death from yellow fever with high virus titers The animals also developed liver lesions grossly similar to those in man and rhesus monkeys and starting with the 4th day many of them exhibited a bright green coloration of the blood serum presumably due to biliverdin The mortality rate was more than 50 per cent higher than for any other African animal including man

TABLE 17

Susceptibility of Galago crassicaudatus lasiotis to Yellow Fever Virus
Quantitative Study of Circulating Virus

Animal no	LD_{50} Asibi virus	Circulating virus as log LD_{50}/cc — Days post inoculation										Serum prot. test postinoculative day
		1	2	3	4	5	6	7	8	9	10	
482	556 000	1 52	4 77	5 02	4 02	2 52						16 *
483	556 000	1 88	5 02	5 02	2 92							16 *
570	460		3 64	7 02	9 88	7 67	died					
571	460		1 52	3 52	7 02	3 67	3 88	1 52				6
639	2 051		1 77	6 28	7 02	3 77	1 52				x	6
640	2 100		4 52	5 52	3 22							6
641	2 100	x	x	x	6 13							6 died 14th day
625	131 000	3 02	6 02	4 39	1 52							died 4th day
638	131 000	x	x	x	1 52							5
642	003	x	x	x	7 16	8 02						died 6th day
615	14 00	2 90	5 52	6 92	6 04	2 00						died 5th day
637	14 500	2 88	5 52	5 06	3 39	1 52						5

* Not tested previously

In the course of these experiments one cycle of transmission was made with *A africanus,* the passage being accomplished by the bites of two mosquitoes infected on a galago 14 days previously The mosquitoes fed readily and the transmission was executed without difficulty That this species does play an active role in the epidemiology of yellow fever was shown by the protection tests on 66 galagos captured in the Gede and Kilifi forests of the Kenya coastal area Nine of the 66 gave positive results indicating that

ever had recently been active among primates of the Kenya coast as confirmed by the positive protection tests found in monkeys *ithecus aethiops johnstoni*) captured in the same forests and by the tration through challenge inoculation that the nine galagos with protection tests were completely immune

ologic examination of the 16 animals that came to autopsy showed lesions that were microscopically different from those occurring in d rhesus monkeys There was widespread necrosis of the liver cells midzonal accentuation but the necrotic cells lacked the marked hilic character of necrotic human liver cells in yellow fever Nuclear ons were not found but there was severe degenerative fatty infiltra all zones of the liver lobule One half of the animals showed gross hage into the gastric mucosa or free blood in the stomach

e present time *G crassicaudatus lasiotis* is the only galago that has ly been shown to play a significant part in the natural cycle of yellow Two of four sera from *E elegantulus elegantulus* captured in the ons gave positive protection tests and antibodies were demonstrated era of numerous monkeys from the same region Therefore it may be ed that *E elegantulus elegantulus* also may enter into the yellow cle but studies on the reaction of the species to virus have not been

galagos thus appear to show a great range of susceptibility to the virus w fever with *G demidovii demidovii* and *G crassicaudatus lasiotis* g the extremes and *G senegalensis* the intermediate type of suscepti All of the positive protection test reactions in properly handled sera to be specific

OTHER AFRICAN ANIMALS

es on African animals other than the primates have been very l Smithburn and Haddow (1949) studied two species of rodents *thus abyssinicus* and *Lemniscomys striatus* sufficiently well to be say that these species show a low level of susceptibility Experiments ll numbers of hyraxes (genus *Procavia*) bush pigs (*Potamochaerus*) and leopards (*Felis pardus*) led to the conclusion that these animals it most only a slight susceptibility to the virus

cold blooded animals were investigated in Uganda by Smithburn fy and Haddow Frogs lizards snakes ghekos and turtles were in

Galago crassicaudatus lasiotis

This species which was studied by Smithburn (1949) in East Africa is the largest of the galagos and comes from the coastal region of Kenya Using Asibi virus Smithburn obtained the circulating virus levels shown in Table 17 In contrast to G *senegalensis,* crassicaudatus had a high proportion of illness and death from yellow fever with high virus titers The animals also developed liver lesions grossly similar to those in man and rhesus monkeys and starting with the 4th day many of them exhibited a bright green coloration of the blood serum presumably due to biliverdin The mortality rate was more than 50 per cent higher than for any other African animal including man

TABLE 17

SUSCEPTIBILITY OF GALAGO CRASSICAUDATUS LASIOTIS TO YELLOW FEVER VIRUS QUANTITATIVE STUDY OF CIRCULATING VIRUS

Animal no	LD50 at birth of mice	Circulating virus as log LD50/cc										Serum first positive day
		Days post inoculation										
		1	2	3	4	5	6	7	8	9	10	
482	556 000	1 52	4 77	5 02	4 02	2 52						16 *
483	556 000	1 88	5 02	5 02	2 92							16 *
5 0	460		3 64	7 02	9 88	7 67	died					
571	460		1 52	3 52	7 02	3 67	3 89	1 52				6
639	2 051		1 77	6 28	7 02	3 77	1 52				x	6
640	2 100		4 52	5 52	3 22							6
641	2 100	x	x	x	6 33							6 died 14th day
625	131 000	3 02	6 02	4 39	1 52							died 4th day
638	131 000	x	x	x	1 52							5
642	003	x	x	x	7 16	8 02						died 6th day
615	14 500	2 90	5 52	6 92	6 04	2 00						died 5th day
637	14 500	2 88	5 52	5 06	3 39	1 52						5

* Not tested previously

In the course of these experiments one cycle of transmission was made with *A africanus,* the passage being accomplished by the bites of two mosquitoes infected on a galago 14 days previously The mosquitoes fed readily and the transmission was executed without difficulty That this species does play an active role in the epidemiology of yellow fever was shown by the protection tests on 66 galagos captured in the Gede and Kilifi forests of the Kenya coastal area Nine of the 66 gave positive results indicating that

yellow fever had recently been active among primates of the Kenya ... This was confirmed by the positive protection tests found in mon... (*Cercopithecus aethiops johnstoni*) captured in the same forests and by demonstration through challenge inoculation that the nine galagos ... positive protection tests were completely immune

Pathologic examination of the 16 animals that came to autopsy show... hepatic lesions that were microscopically different from those occurring ... man and rhesus monkeys There was widespread necrosis of the liver cel... with a midzonal accentuation but the necrotic cells lacked the marked eosinophilic character of necrotic human liver cells in yellow fever Nuclear inclusions were not found but there was severe degenerative fatty infiltra... tion of all zones of the liver lobule One half of the animals showed gross hemorrhage into the gastric mucosa or free blood in the stomach

At the present time *G crassicaudatus lasiotis* is the only galago that has definitely been shown to play a significant part in the natural cycle of yellow fever Two of four sera from *E elegantulus elegantulus* captured in the Cameroons gave positive protection tests and antibodies were demonstrated in the sera of numerous monkeys from the same region Therefore it may be presumed that *E elegantulus elegantulus* also may enter into the yellow fever cycle but studies on the reaction of the species to virus have not been made

The galagos thus appear to show a great range of susceptibility to the virus of yellow fever with *G demidovii demidovii* and *G crassicaudatus lasiotis* forming the extremes and *G senegalensis* the intermediate type of suscepti bility All of the positive protection test reactions in properly handled sera appear to be specific

OTHER AFRICAN ANIMALS

Studies on African animals other than the primates have been very limited Smithburn and Haddow (1949) studied two species of rodents *Arvicanthus abyssinicus* and *Lemniscomys striatus* sufficiently well to be able to say that these species show a low level of susceptibility Experiments on small numbers of hyraxes (genus *Procavia*) bush pigs (*Potamochaerus porcus*) and leopards (*Felis pardus*) led to the conclusion that these animals show at most only a slight susceptibility to the virus

The cold blooded animals were investigated in Uganda by Smithburn Mahaffy and Haddow Frogs lizards snakes ghekos and turtles were in

oculated with varying doses of virus There was no evidence whatever of virus multiplication In Africa as well as in South America the poikilo thermic animals can be excluded from the forest yellow fever cycle

Hedgehogs

Following the demonstration by Findlay Hewer and Clarke (1935) of the marked susceptibility of the Sudanese hedgehog *Atelerix albiventris* (=*Atelerix pruneri pruneri* [Wagner]) to yellow fever virus there was con *siderable interest in the possible epidemiologic* involvement of the Nigerian hedgehog *Atelerix spinifex* (Thomas) a comparatively abundant animal Findlay and Mahaffy (1936*b*) and E C Smith (1936) investigated examples of *the* species from near Kano Findlay and Mahaffy found that the West African species was apparently resistant to the virus there being no reaction in any of five animals inoculated

SUMMARY

The role of vertebrate hosts (other than man) in the epidemiology of yellow fever is complicated and at many points the information available on the subject is inadequate to permit firm conclusions However certain generalizations applicable to both Africa and South America can be made and these are useful in orienting the consideration of epidemiologic proba bilities with reference to the human disease

All of the susceptible animals are characterized by a similarity in behavior following virus inoculation Irrespective of the degree of illness produced there is a preliminary period of clearance of virus from the blood stream so that except with very large doses no virus is demonstrable 24 hours after the inoculation After a period of a few days virus again appears in the blood stream increasing to a maximum and then gradually disappearing The period of circulation varies from 1 to 6 days although occasionally it is slightly longer Coincident with the later phase of virus circulation or be ginning shortly thereafter antibodies appear which are demonstrable both by neutralization and complement fixation tests Accordingly there are at least two moments in the course of an infection when the animal will exhibit the same submaximal titers of circulating virus first during the early phase of virus multiplication and later during the period when the virus titer is falling The titers at these two times may be identical but the conditions with respect to antibodies and infectivity for mosquitoes are quite different

Transmission experiments conducted during the latter phase are l fail even though the virus levels are apparently adequate

Following the disappearance of circulating virus the antibodies esp those concerned with neutralization gradually increase to a maximun a period that is highly variable and may extend to several months The sequent history is likewise subject to differences dependent upon the sp In the primates antibodies persist for life and reinfection probably is n possible A few individuals among some species of marsupials appear experience an actual loss of immunity and can then be reinfected This mo of behavior is exceptional and does not occur with a frequency of any ep demiologic importance

It thus seems to be established that there is no virus reservoir among th nonhuman vertebrates Virus multiplication and circulation is a transien phenomenon lasting for only a few days and leaving an animal refractory to further attack by the virus As in man the persistent antibodies may be used as an indicator of prior infection so that immunologic investigation is avail able as a tool for the epidemiologist The vertebrate host may be regarded as a temporary repository for yellow fever virus and as an agent for the dis semination of virus among the vector mosquitoes which may be considered the true reservoirs

To those who have been intimately concerned with the evaluation of the part played by the various vertebrate species in jungle yellow fever there has come an appreciation of the logical impossibility of generalizing from a comparatively small bit of laboratory investigation In common with all workers in biology students of yellow fever would like to be able to conduct controlled experiments yielding results directly applicable to the field prob lems Too often the experimenter deludes himself with what he calls his controls and fails to realize that the experiment as a whole is not at all con trolled in relation to the conditions obtaining in the forest The conclusion that seemed so tempting and reasonable in the laboratory may turn out to be irrelevant when the same biologic materials are considered in another environment

Complete definition of an ecology involves a quantitative knowledge of many factors only a few of which are ordinarily measured or even recog nized It is probably true that no two habitats are ever identical in all re spects even though separated only by short distances While temperature humidity light intensity and the various gradients of these are customarily measured spatial relationships the floral composition of the forest the

gradients of metabolites such as carbon dioxide and many other factors are also operative None of these environmental complexes is ever duplicated in a laboratory experiment and eventually the investigator must recognize that his controlled experiment permits him only to compare two or more classes under more or less uniform conditions and to reach conclusions that hold for those particular circumstances the findings cannot be extrapolated to other sets of conditions

The demonstration of the susceptibility of an animal in the laboratory consequently tells nothing about its actual part in the natural cycle of the virus It would seem reasonable to assume that an animal species that does not circulate virus under any circumstances in repeated trials and that gives no evidence of virus neutralization is genuinely nonsusceptible and may be excluded from epidemiologic considerations The reverse however is emphatically not true The rhesus monkey for example although highly susceptible has no epidemiologic significance at the present time although it might become important were yellow fever virus introduced into India *G senegalensis* although as susceptible as any African primate does not appear to play a part in the virus cycle of West Africa because it inhabits a savannah forest

The chief problem of the laboratory has been to find some method for detecting the actual involvement of an animal in yellow fever cycles in the forest Practically speaking the only reliable indication of such previous experience is the neutralization test Here again the experience with one particular species cannot be extended to the interpretation of results with an unrelated category of animals direct experimentation to determine the immunologic behavior of each species is essential *Standards for each species must* be established through the study of sufficiently large samples of normal animals and the reactions to contact with the virus must be evaluated against *this standard The differentiation may prove to be relatively easy for some* orders and quite difficult for others The existence of nonspecific virus neutralizing factors in the sera will *make the interpretations more difficult in all cases and in some species* such as the domestic cow and many birds may invalidate the tests completely

If the protection test in a particular species is found to be reliable and specific and thus usable in determining the extent to which the species is naturally involved the epidemiologist can then obtain factual information with which to work The presence of specific antibodies together with an approximation of the age of the animal may serve to reconstruct the pic

ture of past outbreaks of infection among the animals just as accu
as the same procedure may be used among the human population

Other tests such as complement fixation and various forms of agglu
tion such as the collodion pellet test studied by Weir have not been fo
to be as reliable as the protection test especially for survey sera where o
narily the quality of the specimens is not uniformly good The continu
search for new tests however bespeaks the disadvantages of the expensi
and comparatively slow mouse protection test

Immunologic research has made it clear that the vertebrate hosts of yello
fever are restricted to the mammals and that among these the primates are
dominant in both South America and Africa All members of this order ap
pear to be susceptible to the virus of yellow fever although there is a wide
range of variation in the severity of the disease produced This variation is
apparently conditioned upon differences among the animals and upon strain
differences with respect to the virus In general the species most frequently
infected under natural conditions show little sign of disease so that introduc
tion of yellow fever virus produces little or no visible effect on the animal
population

With a population that experiences high fatality when infected rapid
decimation would be expected if the species were involved to any great
extent in the epidemiology hence the coexistence of endemicity and a large
population of nonimmunes of such a species indicates a remote relationship
to the natural cycle although the possibility is by no means excluded This
would appear to be the case with such species as the members of the genus
Aotus in the Colombian llanos On the other hand where such a species
is primarily involved an explosive epidemic may be anticipated with a few
immune survivors as was found at Volcanes

As far as the investigations in Africa have gone jungle yellow fever re
mains a problem purely of the primates and there is as yet no evidence that
any other order is involved The mammalian portion of the cycle thus re
mains essentially simple while the mosquito mechanisms appear to be quite
complex The species in any one region are not of equal consequence (Had
dow Smithburn et al 1947) probably because of the differing degrees of
overlapping of the habitats of the animals and the mosquito vectors

While in South America the primates are again clearly the dominant
order other orders appear to enter the picture to a variable and undeter
mined extent These are the marsupials and some of the rodents In Co
lombia the conditions vary from a high population of monkeys with no

marsupials as in the Macarena range to no monkeys and a high population of marsupials as in Muzo yellow fever has been encountered in both areas Between these two extremes there are all possible gradations The marsupial order shows more variation in susceptibility than do the primates and some species in certain areas appear to be definitely refractory Most of the order however is characterized by some degree of susceptibility

Among the rodents there are a number that are susceptible to the virus but for various reasons they seem unlikely to take part in the forest cycle In some specialized regions particular rodent populations such as the *dasyprocta* may become important

While all of the edentates are susceptible only those that wander about during the daytime such as the sloth and the anteaters appear to become infected The population densities of these are always low so that at best they could play only a secondary part in the cycle of yellow fever

The cold blooded animals and the birds constitute a great category that is definitely not susceptible Despite extensive experimentation no evidence of virus multiplication within these two groupings has yet been reported

7 THE CLINICAL ASPECTS AND DIAGNOSIS OF YELLOW FEVER

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SYMPTOMATOLOGY

Degrees of Severity

The Three Clinical Periods of Yellow Fever

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Mortality

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CLINICAL PATHOLOGY

General Pathology

The Blood

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DIAGNOSIS

Clinical Diagnosis

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Treatment under Primitive Conditions

YELLOW FEVER VACCINATION REACTIONS

Reactions to the 17D Vaccine

Reactions to the French Neurotropic Vaccine

Reactions with the Dakar Scratch Method

YELLOW FEVER is an acute infectious disease of short duration and extremely variable severity, caused by a filtrable virus and followed by life long immunity The classic triad of symptoms—jaundice, hemorrhages and intense albuminuria—is present only in severe infections, which are now known to comprise only a small part of all the infections which occur

The variability of yellow fever was clearly recognized by one observer over one hundred years ago during an epidemic in Mobile, Alabama

> When Yellow Fever shakes off its mild endemic form and assumes that of a great *Epidemic*, as it did here in 1839, it comes robed in majesty and power—all febrile diseases disappear before it, or are compelled to wear its livery—the peculiar characteristics of the disease stand out boldly and with few exceptions, all difficulty of diagnosis vanishes—patients are stricken down by hundreds with attacks varying from the mildest to the most malig nant and yet all wholly unlike periodic fevers—in the same family and house, one will be so lightly attacked as scarcely to lie down, while another is dying with all the horrors of black vomit and what is particularly worthy of note, the light cases pass off spontaneously in two or three days *without a dose of quinine,* and afford *protection against the disease in after years'* (Nott, 1848)

SYMPTOMATOLOGY

As Nott has so vividly indicated, the variation in the severity of attacks of yellow fever during a single epidemic is extraordinary even among members of the same family The range is from a febricula that is absolutely undiagnosable clinically to a fulminant fatal attack with all the classic signs and symptoms of the disease

Prior to the development of the precise laboratory diagnostic measures that the isolation of yellow fever virus made possible, many mild attacks of yellow fever were not recognized Even today there is a widespread tendency to think of yellow fever as a malignant disease that is usually fatal This concept of the disease is wrong and we shall attempt to present the true picture

DEGREES OF SEVERITY

Before discussing the symptoms of yellow fever it is well to classify attacks of yellow fever according to degree of severity (1) very mild (2) mild (3) moderately severe (4) malignant

1 In very mild yellow fever the only symptoms are fever and headache lasting from a few hours to a day or two The disease is clinically undiagnosable even in the presence of an epidemic of yellow fever But a positive diagnosis can be made by means of special laboratory procedures

It is possible for completely inapparent infections to occur especially in endemic areas where as a result of long contact with the virus the population is genetically selected in respect to yellow fever Such infections may occur in babies who are losing the passive immunity bestowed upon them by immune mothers and who are infected with exactly that amount of virus which vaccinates them without symptoms

2 In mild infections the fever and headache which usually begin suddenly are more pronounced Additional symptoms appear nausea epistaxis Faget s sign (relatively slow pulse in relation to constant or rising temperature) slight albuminuria and subicterus The illness lasts only 2 or 3 days and is clinically undiagnosable except during an epidemic more especially where there are other cases in the same household Without studying the patient by laboratory methods the clinician can diagnose such cases only as suspect yellow fever

3 Moderately severe yellow fever is clinically diagnosable because one or more of the classic symptoms is present The fever is higher and Faget s sign is more definite Headache and backache may be severe Nausea and vomiting are more troublesome Definite jaundice and marked albuminuria are present There may even be black vomit or uterine hemorrhages The duration of the fever is from 5 to 7 days

Abortive infections are severe at onset but the patient recovers rapidly usually in 3 or 4 days These infections are an exception to the rule that moderately severe yellow fever has an appreciably longer course than mild yellow fever

4 Malignant yellow fever whether fatal or not presents all the classic symptoms accompanied by a great variety of other symptoms

Fulminant yellow fever is the hyperacute form of the disease resulting

in death on the 3d or 4th day. Often all of the classic symptoms are present.

THE THREE CLINICAL PERIODS OF YELLOW FEVER

Moderately severe and malignant attacks of yellow fever are characterized by three distinct clinical periods: the period of infection, the period of remission, and the period of intoxication (Fig. 49).

During the period of infection, which lasts about three days, the virus is present in the circulating blood, often in large amounts, but this presumably represents merely an overflow from the tissues in which multiplication of the virus takes place. The hyperemia of the skin is an index of the generalized hyperemia which occurs. The patient may be extremely uncomfortable because of severe headache and generalized aches and pains in muscles and joints. *He is usually unable to sleep, overalert and irritable.* The fever continues high at 39 to 40°C. or even higher. The nausea and vomiting are sometimes severe.

Then follows the period of remission indicated by the fall of the temperature to or toward normal (Fig. 49). The patient rather suddenly feels much better, although it is during this period that the prognosis must be most guarded. His headache and other aches are much less severe or even disappear. He is less nauseated and may sleep quietly. This stage lasts from a few hours to a couple of days. The variability of clinical yellow fever being what it is, the period of remission may not be present at all or it may merge into frank convalescence.

The third stage is the period of intoxication. In this stage free virus usually is not present in the circulating blood. On the contrary, sensitive tests have demonstrated the presence of increasing amounts of neutralizing antibody in the blood of a human patient during the stage of intoxication (Berry and Kitchen, 1931). Neutralizing antibody has been repeatedly demonstrated in the blood serum of rhesus monkeys that were dying of yellow fever.

While the virus is gone from the blood, the toxemia it produced remains. The classic symptoms of yellow fever, which are manifestations of this toxemia, become fully developed. The fever rises again, but the pulse remains slow, moderate jaundice becomes evident, vomiting is more troublesome and the vomitus usually contains blood that has been blackened by

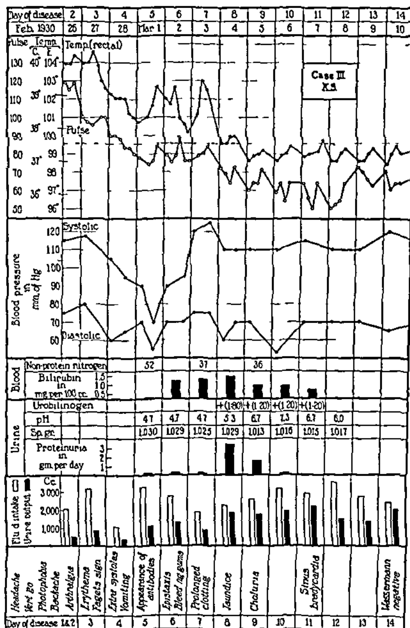


Fig. 49 Clinical events in yellow fever patient (*Berry and Kitchen, 1931*)

the action of the gastric juices, that is, black vomit Albuminuria is always present and may be very intense, oliguria frequently occurs

When it is realized that the toxemia affects the liver, the heart, the kidneys and the blood vessels generally, not to mention the small vessels of the vital centers of the brain, it is to be expected that sometimes the natural defenses of the body will be unable to overcome the deleterious effects of the intoxication, and the patient will die

The period of intoxication is the most variable of the three periods At its maximum, it is much the longest In mild infections it is not recognizable at all When recognizable, its usual length is 3 or 4 days but it may be extended to as much as 2 weeks—in the rather exceptional instances in which an uncomplicated attack of yellow fever is followed either by a long period of asthenia with recovery, or by a late death, probably due to cardiac failure

Lins (1929), who had extensive experience with yellow fever during the 1928–1929 epidemic in Rio de Janeiro epitomized the clinical picture of yellow fever by saying, "the intoxication is everything the infection nothing or almost nothing"

SYMPTOMS

The incubation period, or the length of time elapsing between the bite of an infectious mosquito and the onset of symptoms is commonly accepted as 3 to 6 days Significantly longer incubation periods of 10 and 13 days have been reported (Low and Fairley, 1931) in persons infected in an accidental manner, presumably through the skin but in any event without the aid of a mosquito It seems possible, therefore that the incubation period of an infection acquired in the natural way may sometimes be more than 6 days

Prodromal symptoms are usually absent If present they are very vague and of short duration The patient may experience a very slight feeling of general indisposition for a few hours before onset of fever, or a similar period of very mild stimulation or excitation

The onset is characteristic It is sudden Headache and dizziness develop rapidly while the individual is in the midst of usual tasks or he may awaken from sleep with these symptoms The temperature rises rapidly to 39 or 40°C and the headache becomes more severe Muscular aches and pains become generalized Often there is nausea, with vomiting of food or mucus Small children may experience an initial convulsion

Examination of the patient reveals a flushed face, neck, and upper chest

The conjunctivae are injected The skin is dry and hot Mentally the patient is alert and apprehensive Often he looks as if he were intoxicated with alcohol or he looks and feels as if he had been sitting with his face too close to a very hot fire

The pulse is full and bounding Very early it is more or less proportional to the fever ranging up to 100 or even 120

On the 2d day of fever the general condition of the patient usually remains unchanged The tongue gradually acquires a rather characteristic appearance with bright red margins and tip and a furred center The gums become congested and ooze blood upon slight pressure

Faget s sign which makes its appearance by the 2d day if not sooner is one of the most constant findings in yellow fever if the attack is at all severe

Lack of appetite coupled with thirst is not uncommon Urine is voided in satisfactory amounts rarely does it contain albumin There is usually nothing noteworthy about the bowels

This is the picture of an attack in the malignant the severe and some of the mild cases which will eventually prove to be clinically diagnosable But in the rest of the mild and in all the very mild infections the picture is much less definite

On the 3d day of illness many things can happen The fever may fall rapidly to normal and the patient enter into frank convalescence He may be back doing heavy work a few days later though this is to be avoided if possible In such instances the patient suffers an abortive attack of the disease At the other end of the scale are the fulminant attacks in which on the 3d or early on the 4th day anuria copious hemorrhages from the gastrointestinal tract or wildly agitated delirium—or a combination of the three—supervene and the patient dies

Having mentioned the extremes we may now turn to the more common occurrences of the 3d day which is usually the last day of the period of infection

Jaundice first becomes detectable about the 3d day as a subicterus of the sclerae It seldom appears before the 2d day of fever The earlier jaundice appears the more likely it is to be severe In spite of the name yellow fever jaundice is often not a prominent symptom of the disease even in fatal cases It always develops more or less gradually and may not be severe enough to be noticed by the family of the sick person until after he has died or has recovered from his illness Jaundice of the skin is sometimes very pronounced when death is somewhat delayed or during convalescence

When of clinical degree jaundice varies from a subicterus of the sclerae through a moderate generalized icterus to rarely an intense icterus

The vomiting of blood that has been blackened by the action of the gastric juices is one of the most horrible and characteristic symptoms of yellow fever The Spanish and Portuguese named the disease black vomit with good cause For there is nothing more tragic or more terrifying for the family of a person ill with yellow fever than to watch the sudden vomiting of a huge amount of black vomitus and then collapse and death

Minor hemorrhages may occur early in the period of infection Epistaxis is common at or soon after the onset of fever because of the congestion of the nasopharyngeal mucous membranes If such blood is swallowed flecks of black vomit may appear very early in the disease before there is any bleeding from the stomach Copious epistaxis may also occur during the period of intoxication Early in the disease the gums are hyperemic and may bleed slightly on pressure later they may bleed freely and spontaneously

The bleeding into the stomach occurs from ecchymoses of the mucosa usually in the region of the pylorus The amount of blood in the stomach is sometimes small and the vomitus resembles coffee grounds However large the amount of blood it is almost always much darkened Indeed in the early days before it came to be generally recognized that the black material was really altered blood there was much controversy as to the nature of the vomitus

Melena is a common manifestation of gastrointestinal hemorrhage and it often occurs when there is no black vomit Usually the stool is tarry because the stomach or duodenum is the site of the hemorrhage But red blood is passed when hemorrhage takes place much lower in the intestinal tract as from an ulceration in the large bowel Uterine hemorrhages are not uncommon but macroscopic bleeding from the urinary tract is rare Other sites of hemorrhage are legion particularly if there is some pre existing lesion of the skin or mucous membrane

Early in the course of the disease there is nothing noteworthy about the amount or content of the urine The volume and specific gravity of the urine are closely correlated with the fluid intake Moderate to very intense albuminuria is always found in severe infections but it rarely appears before the 3d day of illness In the classic picture heavy albuminuria develops suddenly within a period of 12 hours the amount of albumin may increase from insignificant traces to quantities such that the urine coagulates in the

ibe when tested for the presence of albumin Albuminuria may last a few days and then disappear almost as rapidly as it appeared

On the other hand the mild and very mild cases are characterized by the relative absence of marked albuminuria There may be a trace of albumin in the urine but no more than would be expected in any patient with a similar degree of fever

There is a rough positive correlation between the severity of the attack and the amount of albumin in the urine If as has been done in some epidemics albuminuria is considered requisite for the clinical diagnosis of yellow fever many mild cases may be missed As with jaundice and hemorrhage albuminuria may or may not be present and if it is present may be either slight in degree or exceedingly intense

Complete anuria is very rare but severe oliguria is often accompanied by paresis of the bladder Catheterization is then necessary to get the 30 to 100 cc of urine that are secreted in 24 hours In such cases the urine is heavy with albumin and often contains casts Oliguria is a phenomenon of the period of intoxication except in fulminant infections when the periods of infection and intoxication are merged

As mentioned above the pulse at onset is full and bounding and the heart sounds are strong and clear The blood pressure is normal or elevated During the period of infection there is not much change except for the development of Faget s sign

A fully developed period of remission is usually accompanied by a marked drop in blood pressure a further slowing of the pulse which becomes thin and weak and often a muffling of the heart sounds

Still later in the period of intoxication the pulse continues slow in rel tion to temperature except that there may be a terminal tachycardia Ofte there is a true bradycardia which occurs independently of the jaund present The pulse is usually weak extrasystoles frequently occur The he sounds are muffled and a variety of anomalous heart sounds may devel When the patient goes into collapse the blood pressure falls but other it is variable

Chagas and Freitas (1929) made exhaustive electrocardiographic stu of 11 naturally infected persons in Rio de Janeiro several of whom of the disease They found a variety of changes in their tracings ative of the wide variety of lesions to which the myocardium i ject but they found no changes peculiar to or characteristic of fever Lins (1929) obtained similar results on a larger number of patie

intensively studied Chagas and Freitas concluded that marked and progressive changes in the T wave were of very grave prognostic import but Berry and Kitchen (1931) found that these same changes were not incompatible with survival in their patients Electrocardiographic studies show definitely that the heart may be involved at the very onset of illness and that in some mild cases the heart may be the chief seat of abnormality while in others it may escape entirely (Berry and Kitchen 1931)

In malignant infections coma frequently sets in sometimes 2 or 3 days before death However some patients do recover after being in coma for a day or two Sometimes coma develops in patients whose kidneys are still functioning adequately then it is clearly hepatic in origin But in most cases the marked disturbance in kidney function makes it impossible to ascertain whether the coma is of hepatic or uremic origin

Hiccough often intractable is a most distressing symptom It may even continue after a patient has lapsed into coma or commence while he is in coma

Shortly before death it is not uncommon for the patient to become delirious and wildly agitated sometimes almost maniacal At other times the mind remains lucid until the very end Not included in this particular discussion is the ordinary delirium that may be expected to occur in some patients with a temperature of 40°C The violent and uncontrollable agitation that sometimes precedes death is most distressing It is one of the manifestations of yellow fever that so often gave rise to panic during epidemic periods Terminal delirium of a quieter type also occurs frequently

The direct cause of the fearsome agitation sometimes observed would seem to be the minute perivascular hemorrhages in the brain particularly in the brain stem first described by Stevenson (1939) This is one of the very few clinical aspects of the disease that have been elucidated since 1915 In recent years however there has been no opportunity to make careful clinicopathologic studies on a group of patients large enough to permit a more precise evaluation of the significance of the lesion

MORTALITY

While it seems probable that the over all fatality rate in yellow fever is between 5 and 10 per cent of *all cases* and not merely of all clinically recognizable cases the rate may rise above these limits in a given epidemic

Recognition of the fact that the mortality from yellow fever is relatively low antedates by many years the perfection of laboratory methods for the diagnosis of the disease. Not long after his service with Walter Reed in Cuba James Carroll (1907) wrote:

> Under favorable conditions the mortality rate from yellow fever should not exceed 10 or 15 per cent; it should never exceed 20 or 25 per cent under any circumstances, and a higher death rate would warrant the suspicion that the milder cases were escaping recognition. Now that the diagnosis of "bilious remittent fever" can be excluded it is becoming more apparent that a fairly large proportion of cases of yellow fever are mild in character and recover without black vomit.

Carroll's statement probably represents the best efforts of a most astute clinician who did not have at his disposal the special laboratory procedures needed in yellow fever.

Carter (1922*a*) on this same subject states:

> Yellow fever is counted, and with reason, a disease of high mortality, and yet this is not true of all epidemics. The variation of different epidemics is so great that it is futile to speak of an average mortality, yet it is fair to say that in the past the mortality recorded has been decidedly greater than the truth. This comes from not recognizing mild cases which naturally recov-ered. Bucaramanga and Cucuta in Colombia in recent years gave about 6 per cent; also in great epidemics. The epidemic of 1897 in the United States gave I think about 6 to 10 per cent mortality, that of 1898 decidedly less—probably 4 to 6 per cent. These, although careful estimates, are estimates only. Yet when, as at the writer's first acquaintance with yellow fever, of young men landing in Memphis, Tenn., July 7, 1879, 6 died before Sep-tember; when a vessel leaving Rio with 26 men arrives at a United Stat port with 10 men alive, one knows that it can be severe. There were 7 8 deaths reported in New Orleans in 1853 out of a population of about 30 0 (Touatre).

The very low mortality rates given by Carter for Cucuta and B manga are of interest in the light of the findings of Kerr and Patino Ca (1933). At Socorro, which is quite close to Bucaramanga, the case f rate was between 1 and 2 per cent in an epidemic which attacked l population of a town of 5,000 people. Furthermore, there were cle cations that the population had never before suffered from epidem fever. There was very little Negro blood in the population. It wou

therefore that the yellow fever virus in the Bucaramanga Socorro area was a type that killed only a very small proportion of the persons infected with it

RACIAL SUSCEPTIBILITY

Man is universally susceptible to yellow fever regardless of race Negroes are reputed to be less susceptible to the disease than are other peoples and it is probably true that the case fatality rate is usually lower among them Nevertheless Negroes do develop severe and even fatal yellow fever

RELAPSES COMPLICATIONS AND SEQUELAE

No clinical relapse of yellow fever has ever been demonstrated The humoral immunity to yellow fever is a very enduring affair but the very fact that neutralizing antibody is present for so long in the blood and in such appreciable amounts has led to the concept of a nonsterile immunity According to this theory virus persists in the body somewhere safely ensconced inside cells where the circulating antibody cannot get at it periodically some of the virus gets out of the cells and stimulates the production of more antibody The process is akin to continual or periodic revaccination

It is theoretically possible for a subclinical relapse of yellow fever to occur This does often happen in malaria when the plasmodium can be found in the blood but the patient has no symptoms of the disease Such a relapse in yellow fever would be characterized by the presence of free or unneutralized virus in the blood Inasmuch as this could well occur without producing symptoms it is most unlikely that opportunity will arise to demonstrate such virus

Clinical yellow fever is relatively free from complications Suppurative parotitis usually unilateral is the most striking complication It is probably associated with the dry mouth and fauces that develop in some patients especially if their fluid intake is low Pneumonia while not frequent can be a serious complication of yellow fever Abscesses of the kidney possibly as a result of catheterization have been observed Gangrene of a foot or part of a leg has been reported as a relatively rare complication

Myocardial failure after apparently full recovery is a definite hazard Kirk (1941) reports several deaths from myocardial failure in African patients who against his advice left the hospital early in convalescence Berry and Kitchen (1931) observed serious damage to the myocardium of one

patient who made a complete recovery after an exceptionally long convalescence Asthenia sometimes very marked may last for a week or so after the temperature returns to normal but cannot be called a true sequela

At the beginning of the twentieth century it was the consensus of the ablest clinicians that there were no late sequelae of yellow fever that recovery to the status quo ante was complete While there have been no opportunities to study an adequate number of persons before and long after their yellow fever infections in the light of present knowledge of metabolism there seems to be no valid reason for thinking that the earlier clinical opinion should be altered

CLINICAL PATHOLOGY

Knowledge of the way physiologic processes are disturbed by the profound toxemia of yellow fever is essential for the rational therapy of the disease Unfortunately this information is either very scanty or completely lacking Twenty years have passed since the last occasion for hospitalization of a significant number of patients under conditions suitable for a detailed study of the disease Lins (1929) and his colleagues made a great effort to learn as much as they could of the disturbances of metabolism that yellow fever produced in their patients in the 1928–1929 epidemic in Rio de Janeiro As Lins himself points out the studies leave much to be desired In the subsequent 20 years however much information concerning the metabolic events taking place in the liver has been obtained and more is constantly being acquired

Should another urban epidemic of yellow fever break out it would be worth while to make an intensive study of the disease based on the most up to date knowledge of physiology and using the most recently perfected techniques It seems certain that careful investigation would lead to the discovery of at least a few remedial measures that might save the patient s life

It may be mentioned that in South America since 1930 many thousands of persons have contracted yellow fever and many hundreds have died of the disease The vast majority of these persons were infected in jungle outbreaks of the disease Most of them took sick and died in their homes which are frequently most primitive structures Others were hospitalized in very rudimentary hospitals and only a minute fraction of the patients could

possibly have been submitted to a careful study—a fraction far too small to permit useful analysis Because of the existence of permanent forest reservoirs of the virus this state of affairs may be expected to continue in South America and probably in Africa

ORGAN PATHOLOGY

Some of the salient points regarding the histologic changes that occur in fatal yellow fever in man may be mentioned here Full details are presented in Chapter 3, Pathology

First of all necrobiosis and acidophilic necrosis of the parenchymal [illegible] of the liver occur in a characteristically discontinuous fashion in the [illegible] zones of the liver lobules The necrosis involves from 5 to [illegible] per cent [illegible] the parenchymal cells of the liver Marked fatty changes in the [illegible] cells of the liver are more extensive than the necrosis

In the kidney the virus produces fatty changes necrobiosis [illegible] of the tubular epithelium The lesion is a nephrosis which [illegible] severe Fatty changes also occur in the cardiac muscle In the spleen there [illegible] necrosis of the cells in the germinal centers The mucosa of the pyloric [illegible] of the stomach and to a lesser extent of the proximal portion of the duodenum is the site of multiple minute hemorrhages into the gastrointestinal tract Although the individual hemorrhages are small the total [illegible] blood lost is sometimes large In the brain there are minute perivascular hemorrhages often so situated as to be incompatible with life (Stevenson 1939)

BLOOD COUNTS

There are many conflicting statements in the literature regarding the total leukocyte and differential counts It seems generally agreed that there [illegible] be a marked terminal leukocytosis in fatal cases At least part of the discrepancies still remaining are attributable to a lack of control observation on the general population from which the yellow fever patients were drawn A person who contracts yellow fever may also be suffering from a variety of other tropical diseases In a group of Nigerian natives Bugher found leukocyte counts of from 7 800 to 19 300 on the 1st to 7th day of illness All patients recovered And the differential counts were within normal limits even the eosinophil counts

Lins (1929) observed leukopenia consistently in a large series of patients

in Rio de Janeiro The patients were of diverse racial and national origins Lins considered leukopenia of great value in the differential diagnosis Berry and Kitchen (1931) made very careful studies of the leukocytic reaction in a small series of nonfatal laboratory infections contracted in New York by otherwise healthy white males aged 21 to 49 years (Fig 50) These investi

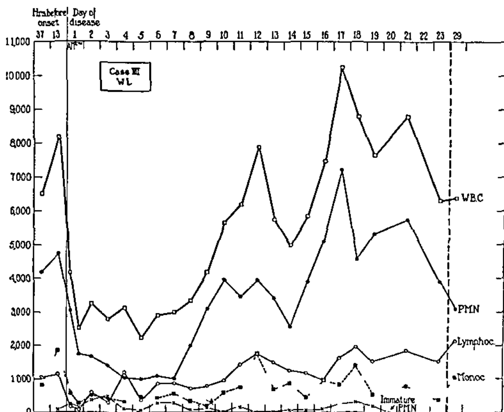


Fig 50 Blood cell counts of yellow fever patient (*Berry and Kitchen 1931*)

gators found a progressive leukopenia early in the disease with a low of 1 500 to 2 500 cells per cu mm on the 5th day of the disease The greatest decrease was in neutrophils By about the 10th day the count had returned to normal with a tendency to rise above normal during convalescence Berry and Kitchen (1931) thought the appearance of the cells to be indicative of some disturbance of the myelogenic functions of the bone marrow

The red cell count and hemoglobin remain within normal limits except sometimes terminally Under these conditions Lins (1929) found counts of over 6 000 000

The platelet count is considered by Lins (1929) to be within normal limits

Bugher found a slight to pronounced increase in the sedimentation rate of the blood in eight African patients ill with yellow fever in Nigeria

Prothrombin time appears not to have been studied in yellow fever However Lins (1929) found variation in the coagulation time of whole blood in a series of eight observations In two patients who had jaundice the coagulation time was much increased Vellard and Vianna (1929) found abnormalities in the coagulation of blood as early as the 2d day of illnes

BLOOD CHEMISTRY

Hypoglycemia is rather to be expected when the liver is severely damaged yet Lins (1929) found no evidence of this condition at any stage of the disease in his appreciable series of naturally infected patients whether or not the individual had received carbohydrate by any route The findings of Berry and Kitchen (1931) in the two patients whose blood they examined were essentially the same Kuczynski (1929) however did find marked hypoglycemia in persons accidentally infected with yellow fever There is need for further study of the glucose content of the blood in human yellow fever

In rhesus monkeys experimentally infected with yellow fever Wakeman and Morrell (1931*a*) found no changes in fasting blood glucose until 24 hours before death At that stage hypoglycemia appeared regularly and increased progressively as death approached After hypoglycemia had appeared little glycogen was found in the livers of the monkeys These changes in carbohydrate metabolism definitely preceded the disturbances of deamination and urea formation also found by Wakeman and Morrell (1930*b*)

Lins (1929) reported the results of nine tolerance tests with levulose and galactose performed on yellow fever patients under a wide variety of conditions The amounts of both sugars in the urine were always well within normal limits In some of Lins s cases there is reason to doubt that the sugar was absorbed from the intestine

Wakeman and Morrell (1931*b*) found that rhesus monkeys ill with yellow fever had diminished tolerance for dextrose These investigators interpret their findings as indicative of loss of hepatic function

The total proteins of the blood serum were normal in three observations by Lins (1929) Apparently no studies of the albumin globulin (A G) ratio

in man have been made, but in rhesus monkeys Wakeman and Morrell (1932*a*) found diminished serum proteins with reduced A G ratios terminally *This is rather to be expected*

Lins (1929) found the total nonprotein nitrogen of the blood markedly increased in six patients five of whom died Urea nitrogen was increased in three patients but creatinine was normal Lins also found amino acid nitrogen much increased especially in severe infections

Wakeman and Morrell (1930*b*) *studied the nitrogen metabolism of rhesus monkeys experimentally infected with the Asibi strain of yellow fever virus This strain has been very extensively used and it produces an overwhelmingly fatal infection in about 95 out of every 100 monkeys It is worthy of note that many of the experiments were done on monkeys fed regularly by tube with liquid diets of known composition* In animals so treated many findings were the same as in animals that did not eat the food offered to them in the terminal stages of the disease

While the results in rhesus monkeys *may not be entirely applicable to* man because of marked differences in the *metabolism of the two species* the findings of Wakeman and Morrell with respect *to alterations in the* blood and urine are of interest

> The nonprotein nitrogen amino acid urea and rest nitrogen of the blood increase by *considerable amounts during the last hours of life*
>
> Blood creatinine uric acid and ammonia change little if at all
>
> Amino acid increases both in absolute amount and in proportion to the nonprotein nitrogen while urea decreases in proportion to nonprotein nitrogen A few cases were found in which there was an absolute decrease in blood *urea nitrogen*
>
> These changes were found *to be terminal events No significant alterations occurred during the early stages of the disease* or in those monkeys which recovered
>
> There were *no marked alterations in the partition* of these substances in the urine
>
> The changes in the *terminal period are shown to be much more pronounced when the accumulation* of these substances in the body fluids is considered
>
> *Organic acid and phosphorus excretion usually increased considerably* during the disease
>
> The changes observed were *interpreted as resulting from a loss of liver function*

No definite evidence of serious impairment of kidney function was observed except a terminal anuria probably due to extreme reduction of blood pressure

The blood guanidine was slightly increased in one patient with a mild infection who was studied by Berry and Kitchen (1931) Findlay and Hindle (1930) found an increased amount of guanidine like substances in the blood of monkeys with yellow fever Berry and Kitchen (1931) suggested that guanidine may possibly be the toxic substance responsible for the gastro-intestinal hemorrhages in yellow fever This interesting matter appears not to have been studied further although Minot (1931) found very definitely increased blood guanidine not only in carbon tetrachloride poisoning with its marked liver necrosis but also in other conditions associated with tissue destruction (Minot and Dodd 1933)

Lins (1929) reported seven determinations of total serum cholesterol all in patients with severe attacks Two of the determinations were made during convalescence The cholesterol was normal or slightly elevated Lins pointed out the lack of serial observations on the same patient and the failure to determine the proportion of esterified cholesterol

Lins (1929) also gave much attention to the plasma electrolytes Unfortunately the useful and simply operated flame spectrophotometer did not exist at that time and sodium was not determined Serum chloride was found normal or elevated in six observations Potassium was elevated in four determinations the values ranged from 27 to 33 mg per cent against a normal of 20 mg per cent Calcium in these same sera was usually decreased values being as low as 5 6 mg per cent against a normal of 10 K Ca ratios in the four cases were from 3 21 1 to 5 35 1 against a normal of 2 1 Further studies especially serial observations on the same patients throughout the course of their illnesses are very much to be desired

Lins (1929) found blood chloride increased in one case and decreased in five and the same with bicarbonate ion In rhesus monkeys Wakeman and Morrell (1932a) found serum bicarbonate usually reduced in the terminal stages of the disease The reduction is due chiefly to accumulation of undetermined acid probably organic and to a lesser extent of inorganic phosphate In monkeys they found no alterations in the concentrations of serum base and chloride or in the urinary excretion of chlorides

The pH of the blood was found by Lins (1929) to be within normal limits 7 29 to 7 51 There was considerable variation in the alkali reserve with a

general tendency toward acidosis though one clear case of alkalosis was found

No liver function tests seem to have been done in human yellow fever patients But in rhesus monkeys with experimental yellow fever Wakeman and Morrell (1932b) found greatly increased retention of bromsulphalein They observed some retention before the monkeys developed fever In a study of infectious hepatitis Labby and Hoagland (1947) used several different tests to obtain an index of liver function measurement of the total serum protein with estimation of the albumin and globulin fractions determination of plasma bilirubin levels the bromsulphalein excretion test and the thymol turbidity test The cephalin flocculation test and the ratio of free to total cholesterol may also be of use Some of these tests done repeatedly might prove useful in yellow fever in assessing the prognosis or the value of therapeutic measures used

THE URINE

The 24 hour urinary volume ranges from normal in mild cases to as little as 30 cc in the terminal stages of an illness characterized by anuria In convalescence from a severe attack there may be polyuria The specific gravity of the urine is greater when the quantity excreted is small or the amount of albumin large

Large quantities of albumin are often present Amounts as great as 20 gm per liter have been found although 3 to 5 gm per liter is more common It is not unusual for the urine to coagulate when tested for albumin Casts especially of the granular type are a frequent finding They are without special significance Occasional red blood cells are frequently observed in albuminous urine Crystals of leucine and tyrosine are not present in the urine of yellow fever patients

The pH of the urine is characteristically low at the height of a severe attack Berry and Kitchen (1931) found values of 4 7 (Fig 49) confirming the similar values found repeatedly by Lins (1929) The pH returns to normal during convalescence and is within normal limits in mild cases Penido (1928) reported that he always found the urine acid to litmus paper but he gave very few details of the therapeutic regime dietetic or otherwise of his patients

Lins (1929) and Penido (1928) found the concentration of chlorides in the urine very much reduced (0 5 to 2 5 gm per liter) in patients who de

veloped albuminuria very early in the disease Penido (1928) observed an exception to the above in a patient who died after an exceptionally long illness (length not stated) the concentration of chlorides in the urine increased before death This finding is of interest because the author found that the concentration of chlorides rose sharply to or above normal as recovery began Penido (1928) concluded that there is a marked reduction in the elimination of chlorides by all routes with consequent retention of chlorides However Lins (1929) found blood chlorides to be quite variable sometimes below sometimes above normal

Lins (1929) and Penido (1928) found significantly different urine urea levels in three types of infections in mild cases (but with albuminuria) and moderately severe cases the concentration increased early in the disease remained high throughout the illness and fell sharply at the onset of convalescence sometimes below normal On one occasion a concentration [illegible] 56 gm per liter was observed In patients with marked digestive [illegible] level tended to be low 2 to 4 gm per liter In a group of patients in [illegible] hemorrhages and jaundice predominated the concentration of urea [illegible] to remain within its normal range Penido (1928) reported that in all but the most severe cases the total daily excretion of urea increased during illness and decreased in convalescence

Penido (1928) found that as a rule uric acid and purine bases (iodine reducing) increased early in the course of the less severe infections In the remainder of infections the increase was slight about 0 8 gm per liter In convalescence there was a decrease In general the values were more or less parallel to the urea concentration curve

Bile pigments were found by Penido (1928) only during convalescence the urine sometimes being bottle-green in color Bile salts appeared sooner than bile pigments but never in the first days of illness Urobilin was present in the urine of all patients in variable amounts Berry and Kitchen (1931) detected urobilinogen in a 1 80 dilution of the urine of one patient

Penido (1928) observed large amounts of indoxyl in the urine of patients gravely ill with large intestinal hemorrhages In mild infections and in severe ones without marked hemorrhages the pigment was present in normal amounts

Lins (1929) stressed the fact that he never observed any edema or ascites in spite of the very severe nephrosis that existed in many of his patients

CEREBROSPINAL FLUID

Lacorte and Villela (1928) studied 10 cerebrospinal fluids from patien ill during the Rio de Janeiro epidemic but did not record details regardin stage or severity of illness In all cases the fluid was under increased tensior One fluid was xanthochromatic all the others limpid and colorless The cel count was normal The Pandy test was always negative but the Nonne Apelt test was always positive sometimes weakly sometimes strongly Bile pig ments and urobilin were not found Glucose was increased in only one case a very severe one Albumin was increased in all cases (25 to 55 mg per cent by the method of Ravaut) Chlorides were normal in one case (72 mg per cent) and elevated in the remainder (77 to 85 mg per cent) Calcium was normal (4.2 to 5.2 mg per cent)

DIAGNOSIS

CLINICAL DIAGNOSIS

A patient with all the classic symptoms of yellow fever that is Faget's sign jaundice heavy albuminuria and black vomit presents no difficulty in diagnosis provided that the attending physician thinks of yellow fever as a possibility More difficult to diagnose are the patients seen early in the course of their illness The milder the attack the more difficult it is to diagnose and many mild attacks are clinically undiagnosable

Diagnosable attacks of yellow fever are characterized by a history of sud len onset with headache and temperature rapidly rising to 39 or 40°C ackache and generalized muscular aches and pains dizziness vomiting of ood There is congestion of the face and the conjunctivae and of the gums he pulse is full and bounding and Faget's sign develops The tongue soon quires its rather characteristic appearance furred in the center with bright l margins and tip

During the first 3 days of illness the period of infection the general con on of the patient is good even though he is very uncomfortable On the day there may be slight leukopenia but no jaundice or albuminuria aracteristic sign of yellow fever is the sudden appearance of large nts of albumin in the urine on the 3d or 4th day and of subicterus of lerae about the 3d day Black vomit on the contrary rarely occurs

as early as the 3d day With the period of remission comes a fall in blood pressure and a deterioration in general condition but the patient usually feels better If the period of intoxication with typical black vomit and marked oliguria develops all the classic symptoms of the disease are present

With modern air transportation it is now possible for a person infected in one of the New World or African reservoirs of yellow fever virus to take sick at almost any place in the world The normal incubation period of 3 to 6 days should be kept in mind and if a patient takes sick with symptoms suggestive of yellow fever within 6 days after a possible exposure to the disease yellow fever should be considered in the differential diagnosis no matter where the patient happens to be

LABORATORY DIAGNOSIS

There are three well established procedures for the laboratory diagnosis of yellow fever Two more the complement fixation test and the precipitin test are theoretically useful but have been little used Four of the five procedures require special facilities and the two most useful require considerable time making them of much more value to the epidemiologist than to the clinician

The well established procedures are (*a*) the isolation of the virus from the blood of a patient early in the disease (*b*) the demonstration of the development of the specific neutralizing antibody of yellow fever virus in the blood serum of a patient during an illness and (*c*) demonstration of the histopathologic lesions of yellow fever in a piece of liver obtained after death of the patient either by means of the viscerotome or at autopsy

To isolate the virus from the blood of a person ill with suspected yellow fever the blood should be taken as soon as possible after the onset of fever preferably during the first 3 days However Bugher isolated virus a few times on the 5th 6th and even 7th days after onset of fever The virus of yellow fever has been on one occasion isolated from the blood of a patient 4 hours before the onset of fever

The persons taking and handling such blood should be very careful not to contaminate their skin with it The usual sterile precautions are greatly facilitated by the use of vacuum syringes of the venule type The serum should be tested as soon as possible The specimen should be kept as cool as possible en route to the laboratory where the examination is to be made but it should never be frozen unless it has been possible to decant the serum

from the clot and discard the latter The less hemoglobin in the serum, the better the quality of the specimen At the laboratory (which may be quite a modest affair located in the field), the serum is inoculated subcutaneously *into a nonimmune monkey, or intracerebrally into white mice* of a highly susceptible strain

Any available species of primate can be used provided that the test animals are known to be nonimmune to yellow fever Rhesus monkeys, however, are the most satisfactory primates to use because they are the most likely to develop a febrile reaction while virus is present in their circulating blood But since even a rhesus monkey may not show any rise in temperature, it is wise to bleed each test monkey each day and inoculate its blood serum intracerebrally into mice If the monkey dies, it should be autopsied and a piece of its liver preserved for histopathologic study, regardless of how typical the gross appearance may be When the monkey dies precipitately, passage may be made to other monkeys or to white mice, using blood serum or liver emulsion If the monkey survives, some of its blood serum *should be preserved for examination by the neutralization test along with* the preinoculation specimen, which will have been taken for reference Should the histologic lesions of yellow fever be present in the liver of the monkey, a diagnosis of yellow fever can be made Even so, it is well to have immunologic confirmation

When mice are used for test purposes the undiluted serum is inoculated intracerebrally into a group of six mice It is wise to inoculate a 1 10 dilution of the serum into a second similar group of mice Both groups are examined for symptoms of encephalitis daily, or twice daily, preferably for a month, always for at least 15 days Subinoculation into fresh mice is made with the brain suspensions of any mice that sicken during the period of observation, unless the illness is obviously due to a bacterial contaminant such as salmonella

Once a virus has been established by two or three subinoculations it is necessary to submit it to a specificity test in order to prove that it is the virus of yellow fever and not some other neurotropic virus Such a test is done by making a suspension of the brains of mice ill or dying of encephalitis caused by the unknown agent, diluting the suspension in a suitable diluent, and then mixing aliquots of the material with a known normal serum and with a known immune serum The mixtures are then injected into two groups of mice If the mice that receive the mixture of virus and known normal serum die *while those which receive the known immune serum live* clear

immunologic evidence is obtained that the virus in question is yellow fever virus.

The reason for testing the serum in a 1:10 dilution as well as undiluted is that the undiluted serum sometimes produces nonfatal encephalitis in the first passage mice, whereas the same serum diluted tenfold or sometimes even more produces fatal encephalitis under the same conditions.

The failure to isolate virus from the blood serum of a suspect case, even though the specimen is apparently taken at the proper stage of the disease and is properly handled afterwards, does not prove that the case was not one of yellow fever.

The double neutralization test, or the neutralization test on paired sera, is the second method for diagnosing nonfatal yellow fever, even in an attack of the mildest type. The test requires the collection of two specimens of blood serum, the first as early in the course of the disease as possible, the second 2 weeks or more after onset. Neutralization tests for yellow fever are done in mice on the two sera.

If the first specimen is taken during the first 3 days (72 hours) after onset of illness, it will almost certainly give a completely negative result. If such a specimen is available, the second specimen may be taken as early as the 7th day after onset.

If the patient is not seen until the 5th or 6th day of illness, it is not too late to take the first specimen. Under such conditions the second specimen may be taken 3 or 4 weeks, instead of 2, after onset. Inasmuch as the first specimen may contain a small amount of neutralizing antibody, it is important that the two specimens be tested in mice at the same time. If the results are at all doubtful, *each of the sera should be retested by methods* appropriate for the quantitative estimation of antibody content.

If the first specimen of serum contains no neutralizing antibody, while the second specimen does contain it, positive proof is at hand that the patient has had yellow fever in the interval. Equally conclusive is the presence of much more antibody in the second serum than in the first.

If a patient is first seen when convalescent, it is still worth while to test a specimen of serum. If it contains no antibody, there is proof that the illness was not yellow fever. However, if the serum is positive for antibody, all the neutralization test indicates is that at some time in the past the patient suffered from yellow fever. It does little good to titrate the antibody content of such a serum because antibody titers are not well correlated with recentness of immunization.

Viscerotomy, or the routine removal of a piece of liver for histologic examination from the bodies of all persons in a given area who die of illnesses lasting 10 days or less, can be looked upon as another diagnostic method. The simplicity of this procedure is one of its great virtues. The immunologic methods are all rather cumbersome. By viscerotomy yellow fever has been unequivocally diagnosed in many unexpected places. And the histologic diagnosis has been repeatedly confirmed by immunologic procedures. At other times, when the symptoms during life fully warranted consideration of yellow fever in the differential diagnosis, histologic examination of the liver has served to exclude that disease as the cause of death.

But the histologic diagnosis of yellow fever from liver specimens collected by viscerotomy is not absolutely infallible, even in the hands of pathologists thoroughly familiar with yellow fever. Very occasionally a case of acute yellow atrophy of the liver can be confused with yellow fever. While the gross and microscopic lesions of yellow atrophy of the liver are usually very different from those of yellow fever, the histologic pictures in the liver may sometimes be so similar as to be indistinguishable. And it may be recalled that acute yellow atrophy of the liver is world wide in distribution.

The diagnosis of yellow fever that is based on examination of a single liver specimen obtained in a new area, or under conditions significantly different from those in which yellow fever is known to exist, should be announced with caution. At the same time such a finding is cause for the most urgent and most exhaustive epidemiologic investigations possible. Indicated control measures should be instituted with all possible speed, but not before material for immunologic studies has been collected from the family and associates of the deceased.

Because it is much less sensitive than the neutralization test, the complement fixation test for yellow fever has not become an accepted procedure either for clinical diagnosis or epidemiologic study. A variety of antigens has been used at different times since 1929, the preferred source material at present being brain tissue of mice dying of yellow fever encephalitis.

The greatest usefulness of the complement fixation test would appear to be distinguishing between old and recent naturally acquired immunity and between immunity resulting from vaccination with 17D virus and naturally acquired immunity.

In regard to the latter, the results obtained by Lennette and Perlowagora ( ) are rather different from those obtained by Perlowagora and Hughes. However, the mouse brain antigens used in the two studies were pre

pared in different ways The former authors found that about one sixth of the sera of persons vaccinated with 17D vaccine 2 months previously contained complement fixing antibody while the latter obtained one positive result among 67 sera from persons vaccinated 2 to 3 months previously Both of these studies were done on sera that had been stored under refrigeration in the liquid state Recent work with the complement fixation test in other neurotropic virus diseases indicates that it is important to keep the sera solidly frozen until they are tested

The precipitin test may be mentioned as an incompletely explored possibility Hughes (1933) working with rhesus monkeys developed a precipitin test using selected acute phase and convalescent rhesus sera An in vitro test of this sort might be very useful for the rapid diagnosis of the more severe human yellow fever infections but the merits of this particular test have never been assessed

DIFFERENTIAL DIAGNOSIS

While any one case of yellow fever may be so mild as to be absolutely undiagnosable except by special laboratory procedures or be so blatant that any tyro can recognize it it is equally true that among any appreciable number of cases say 20 or more there will be some that will be accurately diagnosible on clinical grounds alone In fact the history of a fatal illness obtained from a relative or friend who cared for the deceased is often so definite that a presumptive diagnosis of yellow fever can be made

During an epidemic of yellow fever every acute febrile illness should be suspected of being yellow fever especially if there is headache Yellow fever virus has been isolated from a child at play and from an adult whose only symptoms were slight headache and a low fever neither of which lasted more than a day Many acute febrile illnesses will be obviously not yellow fever As to the remainder rapid improvement following the administration of one of the antibiotics now available proves nothing because the patient could have had either some other infection that was cured by the antibiotic or yellow fever so mild that the attack was over in a day or two

Following are some diseases or conditions that in some respects simulate yellow fever and give rise to confusion in diagnoses Infectious hepatitis formerly known as catarrhal jaundice is characterized by fever followed by jaundice The jaundice appears suddenly only after the temperature has returned to normal Anorexia is a very marked symptom *Clay colored stools*

and bile stained urine accompany development of the jaundice The jaund is usually much more intense than in yellow fever and it lasts much long There is little or no albumin in the urine The liver is enlarged and tend at the onset of jaundice decreasing in size as the patient improves

Serum hepatitis has much the same symptoms and range of severity as in fectious hepatitis except that fever is rare in the milder forms The diseas is remarkable because of its very long incubation period of from 2 to months

Acute yellow atrophy of the liver when fulminantly fatal may be ac companied by high fever vomiting even vomiting of changed blood rapidly developing icterus and diminishing output of urine The liver is normal or even much reduced in size In the subacute forms of liver atrophy the much longer course of the disease the irregular and somewhat lower fever and often the very intense icterus clearly distinguish it from yellow fever

Infectious hepatitis and acute yellow atrophy of the liver occur all over the world in yellow fever areas and outside of them Severe forms of the disease have caused confusion with yellow fever (Hudson 1931*a*) Sometimes several fatal cases occur in the same community over a relatively short period giving justifiable cause for concern especially when clinical facilities are meager Histologic examination of liver tissue obtained postmortem usually permits a definite diagnosis to be made Punch biopsies of the liver seem contraindicated because there are less hazardous ways of making a definitive diagnosis

Leptospirosis (Weil s disease) when very severe may closely simulate yel ow fever even to the appearance of black vomit The onset however is sually much more gradual the fever continuous the jaundice more severe nd the albuminuria less severe and slower in its development

Carbon tetrachloride poisoning when severe enough for jaundice to oc r may be accompanied by fever considerable vomiting and marked al minuria The patient may have taken a vermifuge a day or two preceding onset of symptoms in which case the composition of the preparation can ally be ascertained There may be a history of contact with the chemical dustry or as a household cleaning fluid

ngue may simulate mild yellow fever so closely as to be indistinguish xcept by immunologic tests The albuminuria is very much less in e and jaundice is a very rare symptom The rash of dengue should uish it from yellow fever In the New World dengue does not occur

except in the presence of *Aedes aegypti* so a history of contact with that mo-
quito is essential for the diagnosis of dengue

Tick borne relapsing fever which occurs in northwestern South America may also simulate yellow fever but the continuation of fever for a week or so without remission the rapid pulse and the leukocytosis distinguish the condition clinically The finding of the causative spirochetes in the patient s blood and his prompt improvement after a single dose of neoarsphenamine or penicillin confirm the diagnosis

When the simple facilities needed for the laboratory diagnosis of malaria are available, the differentiation of malaria from yellow fever is usually easy unless the patient is suffering from both diseases at the same time Mild parasite relapses are apt to occur in patients with chronic malaria while they are suffering from any febrile disease Sometimes the diagnosis of yellow fever is excluded by the response of the patient to antimalarial therapy

Yellow fever and malaria often occur together in remote places and it is not always feasible for the doctor to carry with him the equipment necessary for the examination of stained blood films The patient may have taken some antimalarial drug but still have some fever The presence of an enlarged spleen indicates merely that the patient has chronic malaria but not that his fever is due to a relapse Under such conditions it is not possible to be sure whether the illness is a mild attack of yellow fever or a mild relapse of malaria

The differentiation of the grave forms of malaria from severe yellow fever is usually clear because the malaria patient is free of jaundice hemorrhages and very marked albuminuria In severe malaria there is usually pronounced anemia accompanied by a subicterus only very occasionally is there frank jaundice

Blackwater fever is characterized by continued high fever prostration and hemoglobinuria without marked albuminuria These features combined with a history of chronic malaria and of recent medication with quinine or atabrine should serve to distinguish blackwater fever from yellow fever

Influenza may well be confused with mild yellow fever especially if the in-
lammation of the upper respiratory tract is minimal and pneumonia does
ot develop There is leukopenia in both diseases but in influenza the pulse
te is increased in proportion to the temperature Unless the urine is ex-
ined daily for albumin the characteristic albuminuria of yellow fever
y be missed A classic attack of yellow fever is clearly different from any
own type of influenza

Before the rash of smallpox appears the disease may be difficult to distinguish from yellow fever because of the flushed skin intense backache and high fever

DIFFERENTIAL DIAGNOSIS UNDER PRIMITIVE CONDITIONS

Nowadays practically all yellow fever occurs in persons who live in primitive tropical conditions This makes the differential diagnosis more difficult for three reasons clinical facilities are often very meager other known tropical diseases may confuse the picture and the patients are doubtless exposed to febrile diseases of completely unknown etiology

The basis for the last statement is that two new viruses the West Nile virus and the virus of Bwamba fever have been isolated from Africans suffering from febrile diseases suspected of being yellow fever In addition several previously unknown neurotropic viruses have been isolated from wild caught mosquitoes in South America and in Africa

In Africa language difficulties may make it impossible to obtain an accurate history Apprehension on the part of a patient may increase the pulse rate to a marked degree thus masking Faget s sign which is so important in the diagnosis of yellow fever

PROGNOSIS

In the majority of cases of yellow fever—the very mild and most of the mild ones—there is no occasion to make a prognosis because the illness is undiagnosed if not undiagnosable

When the patient is ill enough at the onset of disease for the physician to recognize the disease and make a diagnosis the prognosis must always be very guarded On the other hand one must never give up hope no matter how gravely ill the patient is because miraculous recoveries do sometimes occur

During the period of infection the first 3 days of illness deaths are very rare On the 4th day deaths occur from fulminant attacks of the disease The higher the fever and the earlier the appearance of jaundice and of albuminuria the graver the prognosis The subjective improvement during the period of remission must be completely disregarded for the first 2 days but thereafter if the signs and symptoms of the period of intoxication do not appear the patient is on the way to recovery

When there is a definite period of intoxication with a secondary rise of fever the attack must be classified as a severe or malignant one The prognosis is grave because about half of these patients die The most serious symptoms during this period are increasing oliguria wild agitation delirium and coma A pulse rate of 100 or more is a grave sign over 120 very grave Black vomit in spite of its horridness is not overly serious The appearance of hiccough in a comatose patient is of the very gravest import There is a definite positive correlation between the degree of jaundice and the severity of the disease but some patients with intense jaundice do recover

Favorable signs are continued good diuresis regardless of the amount of albumin in the urine and mental lucidity although some patients are perfectly lucid until a few minutes before death The degree of albuminuria is of no prognostic value

Occasionally but very rarely a patient comes out of coma as if by crisis and makes an uneventful recovery

Most deaths occur between the 5th and 9th day of illness After the 10th day deaths from uncomplicated yellow fever are very rare Most late deaths are probably attributable to overstraining a damaged myocardium

TREATMENT

At the present time as in the past the treatment of yellow fever is on a very unrewarding and unsatisfactory basis There is no known specific drug for yellow fever nor is homologous or heterologous immune serum of any curative value It appears that none of the antibiotics has been used in the treatment of yellow fever Most of the patients make a rapid and complete recovery no matter what is—or is not—done for them On the other hand nothing that the clinician is able to do appears to help appreciably in most of the severe infections There is left only a very small fraction of all cases in which supportive treatment combined with careful nursing may possibly save the life of the patient Luns (1929) found no active therapeutic measure to be of value in the considerable number of patients that he treated in Rio de Janeiro in 1928–1929 and he concluded his paper on an exceedingly pessimistic note

The dearth of information regarding deviations from the normal metabolism and nutrition in yellow fever has already been pointed out Much

more *knowledge* on these *matters* is essential if the treatment of the disease is to be placed on a rational basis

DIETARY PRINCIPLES

There is grave doubt that the starvation diet that has been considered advisable in yellow fever is the diet best for the patient The rationale of that diet is purely empirical The patient is nauseated and vomiting there fore give him a liquid diet practically free from proteins and fats and with much less carbohydrate than he needs to maintain body weight There is more than a chance that this treatment is a late relic of the starve a fever stuff a cold school of therapy

Recent studies on the therapy of infectious hepatitis have revolutionized the dietary treatment of that disease Hoagland Labby et al (1946) began their paper with a review of the situation regarding infectious hepatitis

> Increasing attention has been given in recent years to the dietary treatment of diseases of the liver Information supplied from the growing fields of nutrition and metabolism has resulted in the abandonment of nearly all the older methods employed in the therapy of hepatic insufficiency and in the adoption of numerous new measures which in many instances have provided a sharp contrast to those which were formerly in use Proof of the essentially rational nature of the concepts which form the basis of current therapy in diseases of the liver has been afforded by the achievement of increasing success in a field of medical endeavor which was once outstanding for its spectacular failures
>
> The discovery by Goldschmidt Vars and Ravdin (1939) of the value of protein in protecting the liver from damage by hepatotoxic agents en couraged the inclusion of protein rich foods in the diet Since that time diets high in protein and carbohydrates have been almost universally accepted as optimum for repair of the liver although results secured by Mann in the treatment of experimentally induced liver disease in animals indicate that the type of protein in the diet may be highly important

While it is obvious that many things can be done for a patient with infec tious hepatitis that cannot be done for a person severely ill with yellow fever because of the much more acute nature of the latter disease lessons learned from infectious hepatitis may have application in yellow fever

The treatment of yellow fever is discussed under two headings generally

accepted measures and experimental measures that seem logical in the light of present knowledge but that are not of proved value

ACCEPTED THERAPEUTIC MEASURES

Complete rest in bed until the patient is well on the way to recovery is of value because it lessens his nutritional requirements and spares his liver kidneys and heart In addition there is convincing evidence that in healthy individuals the flow of blood through the liver is very much greater when the subject is lying down than it is when he is erect

The comfort of the patient is to be considered because other things being equal the more comfortable he is the quieter he will be and the less food he will need The usual procedures of sponging with cool water to reduce high fever and of cold applications to the head and abdomen to relieve pain and vomiting are indicated Bland enemas would seem to be better for the relief of constipation and flatulence than purges even the single mild laxative that tradition has allowed at the onset of illness The severe headache and myalgia are usually not eased by aspirin In such cases small doses of codeine or demerol may be indicated just to let the patient rest The nausea and vomiting are sometimes alleviated merely by allowing the patient to suck chips of ice The older authors prescribed spoonfuls of thoroughly iced dry champagne or of dry white wine Iced ginger ale of the pale dry variety should be equally satisfactory In more stubborn vomiting procaine by mouth has been found to give relief as has cocaine

An adequate intake of water should be maintained If enough fluid is not taken or retained by mouth the additional amounts should be supplied by parenteral routes

The routine administration of alkali is indicated because impairment of the alkali reserve has been observed in the great majority of severe infections The water given by mouth should be alkaline Citrus fruit juices may be given since they are alkaline in their ultimate effect Alkali should be given intravenously if insufficient quantities are taken by mouth

Glucose is known to be of benefit in hepatic intoxications If adequate amounts of carbohydrate are not taken or retained by mouth glucose should be given intravenously Berry and Kitchen (1931) gave their patients up to a liter a day of citrus fruit juice making use of the carbohydrate and alkali components of that nutriment which is also high in potassium and low

in sodium content Judgment should be reserved about the wisdom of ing increased amounts of potassium to yellow fever patients

Concomitant malaria can safely be treated with quinine There is n ing published regarding the use of the synthetic antimalarial drugs patients with yellow fever

EXPERIMENTAL MEASURES

Experimental therapeutic measures not of proved value and some o them expressly interdicted in the older literature comprise the following

An ample balanced diet of easily digestible foods seems indicated for such a diet contains the basic materials which the liver needs for its detoxi cating activities The first item would be milk preferably boiled Easily digestible carbohydrates are important as are meat broths Chicken and cheese merit consideration

Casein hydrolysate or hydrolysates of other good proteins may logically be administered early and routinely by the intravenous route if the patient does not retain enough of the food taken by mouth Choline and methionine appear to have no advantages over casein hydrolysate

Calcium gluconate may be given intravenously in case milk cannot be retained by mouth It is surely indicated if the serum calcium is low or if guanidine or guanidine like substances are found in the blood in increased amounts

Vitamin K would seem indicated in view of the lengthened clotting time of the blood that has been observed in yellow fever infections however the vitamin might not be beneficial because the liver cells were unable to utilize it

The above measures may be looked upon as supportive therapy during the period of infection If the patient is not seen until the period of intoxi ation has set in they are still indicated and some additional emergency measures deserve consideration

Transfusions of human blood plasma may be of value if the patient is collapse

Should it be established that serious derangements of the electrolytes of e blood characteristically occur in yellow fever appropriate measures to rect such derangements might be instituted

Vhile it is fervently to be hoped that yellow fever may never again be demic in urban populations such epidemics will be possibilities as long

as *A aegypti* is present in tropical and subtropical cities The cities of t southern United States and of India are examples of places in which urba epidemics of yellow fever could occur if the virus were introduced into th human population

If such an epidemic should occur it would seem justified not to adopt the very pessimistic attitude about treatment that has been current up to the present time Rather let the treatment of severe yellow fever be under taken with renewed hope making use of all the knowledge available but employing the strictest possible criteria in evaluating the results obtained

TREATMENT UNDER PRIMITIVE CONDITIONS

If you find a case of yellow fever don t move him Put a tent over him if necessary but care for him where he is was the advice given by Dr Joseph H White when the author began working with yellow fever in 1926 This is still good advice for by the time an illness is clinically diagnosable as yellow fever it is most unwise to require that the patient exert himself Unless he can be moved to a good hospital with minimal effort it is better to leave him in his home even though there he can receive only the most rudimentary care If the patient is in a place where he is likely to be bitten by mosquitoes capable of transmitting yellow fever he should if possible be kept in a tightly screened room or under a bed net during the first 3 to 6 days of fever

Each year it becomes less likely that any large number of patients with yellow fever will be treated in well equipped hospitals In the future most yellow fever will probably occur under primitive conditions More often than not the patient will be found sick in his home where good medical care will probably not be available and there will be only the members of his family to nurse him

All that can be done under such conditions is to advise that the patient stay where he is that he keep as quiet as possible that he drink plenty of water that he suck all the oranges he wants but that he eat moderately as long as his fever lasts If no citrus fruits are available pinches of sodium bicarbonate should be added to his drinking water No alcohol should be given Finally he should be told to be in no hurry about going back to work when he feels better and to be careful about overeating during conva lescence

Kirk (1911) had occasion to supervise the treatment of many hundreds of

African patients under very primitive conditions during the extensive 1940 epidemic in the Nuba Mountains Anglo-Egyptian Sudan He provided his native dressers with glucose or sugar to feed to patients with aspirin to relieve headache and with Sternberg's mixture (sodium bicarbonate 150 grains and mercury bichloride $^1/_3$ grain per quart) to administer for the relief of vomiting He detected no harmful effect from this last and found that it often stopped the vomiting

Even without any treatment or with the exceedingly primitive supportive treatment outlined above one half to two thirds of the patients whose yellow fever is severe enough to be clinically diagnosable will recover Needless to say some of them will die

In an epidemic of jungle yellow fever in the New World the cases of the disease are often widely scattered Frequently only one person in a household becomes infected but that one may be the breadwinner The simple advice outlined above can be amplified for any doctors the community may have It is equally within the comprehension of the intelligent laymen of the community the parish priests the nuns in the primitive hospitals that may exist the larger landholders and more important business men and the engineers in charge of road or railway construction work or petroleum exploration and development projects

YELLOW FEVER VACCINATION REACTIONS

Community prophylaxis against aegypti transmitted yellow fever may be achieved through the control or still better through the eradication of that mosquito When the yellow fever is of the jungle type individual prophylaxis is attainable by vaccination and must be relied upon exclusively

The three different types of vaccine that have been used for large scale immunization have been described in previous chapters In this chapter the unsatisfactory results and the untoward reactions that have been reported following the use of the several vaccines will be discussed

The normal reaction of man to a subcutaneous inoculation of 17D vaccine is perhaps the mildest reaction to any known vaccine This extremely slight reaction to a phenomenally effective vaccine which is at the same time a living vaccine that infects its recipient is one of the happiest paradoxes of modern medicine It is most unusual for a person vaccinated for yellow fever to be sick enough to go to bed or to lose any time from his work (Smith Penna and Paoliello 1938)

REACTIONS TO THE 17D VACCINE

Inasmuch as 17D vaccine consists of the tissue juices of chick embryos i is somewhat surprising that foreign protein reactions to the vaccine occur very infrequently Furthermore they tend to be mild consisting of urticaria or of vague ill feeling which lasts from a few hours to a day or so They de velop within a few hours after the administration of the vaccine There are only a few published reports of more serious reactions Sulzberger and Asher (1942) reported three cases of urticarial and erythema multiforme like skin eruptions following the use of different lots of vaccine Swartz (1943) de scribed a violent allergic reaction that developed a few minutes after the patient received cholera vaccine and yellow fever vaccine The man had angioneurotic edema marked urticaria gastrointestinal symptoms and se vere dyspnea Upon subsequent study he was found to be extremely sensi tive to egg and chicken muscle proteins Sprague and Barnard (1945) de scribed one patient who developed severe asthma edema of the face and generalized urticaria within 15 minutes after receiving yellow fever vaccine The patient gave a history of eczema and asthma and of marked sensitivity to egg white

Fox Lennette et al (1942) have described the encephalitic reactions that followed the use of a particular substrain of 17D for mass vaccination in Brazil Among some 55 000 persons vaccinated with this substrain of 17D 273 or 0 5 per cent were known to have developed symptoms explainable on the basis of an encephalitic process The great majority of symptoms were mild but one child died Laboratory studies on the substrain revealed that it produced symptoms of encephalitis in 28 per cent of the 60 rhesus monkeys used to assay the quality of the vaccine whereas the previously used sub strain of 17D produced encephalitic symptoms in only 5 7 per cent of monkeys similarly inoculated

Attention was first called to hepatitis as a complication of yellow fever vaccination by Findlay and MacCallum (1937b) By far the most serious accident with 17D vaccine was the widespread epidemic of hepatitis in United States Army troops early in 1942 following the use of certain lots of 17D vaccine prepared in the Yellow Fever Laboratory of the Interna tional Health Division (Sawyer Meyer et al 1944) Incomplete totals mounted to 28 000 cases with 62 deaths from acute or subacute yellow

atrophy of the liver (Jaundice following yellow fever vaccination J A M
1942)

The jaundice episode in United States troops was preceded by a simil
episode in Brazil which has been reported by Fox Manso et al (1942
The Brazilian episode followed the use of certain lots of vaccine prepared
in the laboratory of the Yellow Fever Service in Rio de Janeiro Among
19 000 persons vaccinated with two contaminated lots of vaccine 886 were
known to have developed hepatitis 3 to 4 months later and there were 22
known fatalities from so called liver atrophy

It seems clear that in both instances the icterogenic agent was introduced
into the vaccine via the human blood serum that had been used in the
manufacture of the vaccine It was eventually found possible to prepare sat
isfactory vaccine without adding anything except sterile distilled water
There has been no trouble with hepatitis in the several millions of persons
vaccinated since human serum was omitted from the vaccine

REACTIONS TO THE FRENCH NEUROTROPIC VACCINES

Laigret s French neurotropic virus vaccine was the first to be administered
on an extensive scale The virus used was the neurotropic derivative of the
French strain in its 130th to 185th passage The amount of living virus
inoculated was always small though it was prepared in different ways at
different times No immune serum was used Laigret (1937) reported that
in some 21 000 persons vaccinated there were eight serious reactions with
symptoms of alarming involvement of the central nervous system All pa
tients recovered completely without sequelae as far as is known

Mathis Durieux and Mathis (1936) reported the details of vaccination
reactions in 376 persons No reaction was noted in 226 persons or about 60
per cent The authors found yellow fever virus circulating in the blood in
one of these persons Postvaccination reactions occurred in 150 persons or
about 40 per cent These were classified as follows (a) slight reactions—
malaise but no fever—in 67 persons (b) moderately severe reactions—fever
to 39 5°C headache and backache lasting up to 3 days and occurring 6 to
days after vaccination—in 49 persons (c) prolonged reactions in 34 persons
sufficiently severe to be classified as untoward The symptoms indi
involvement of the central nervous system but not to an alarming de
The symptoms appeared 16 to 21 days after vaccination sometimes
the preliminary early febrile period

The use of the Laigret vaccine was limited and quite properly so to p
sons who were exposed to a definite risk of yellow fever Under such con
tions the chance of contracting fatal yellow fever was appreciably great
than the chance of a seriously untoward reaction to the vaccine

REACTIONS WITH THE DAKAR SCRATCH METHOD

Peltier (1918) summarized the results of 20 million vaccinations by the Dakar method in French West Africa In approximately 17 million of these vaccinations a mixture of yellow fever and smallpox vaccines was used

Two types of reaction occurred an early one 5 or 6 days after vaccination consisting of fever backache and headache and lasting a day or two in 10 to 15 per cent of persons vaccinated and a delayed one 12 to 15 days after vaccination consisting of a grave meningo encephalitic syndrome lasting 5 or 6 days The severe reactions were fortunately quite rare Recovery appeared to be complete and without sequelae No fatalities were reported

Enough severe reactions have occurred in white infants to lead Peltier (1918) to recommend that this vaccine not be administered to infants who are in poor health or who have just recovered from an exanthematous disease

8 EPIDEMIOLOGY

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IN DEFERENCE to custom the term epidemiology has been used for the hea ing of this chapter but as applied here it requires broadening qualificatio In the strict etymologic sense epidemiology refers to the study of epidem among human populations By common usage it has been extended to i clude the endemic and sporadic occurrence of disease and all those facto that contribute to its spread and persistence The term has also been used connection with disease of lower animals and even plants Whatever may the justification of this usage its meaning in connection with yellow fev must embrace infection by the virus of all known susceptible hosts inclu ing both vertebrates and arthropods as the disease in man is essentia linked to the life history of the virus in these extrahuman hosts

The epidemiology of an infectious disease may be said to rest upon a tri —the infectious agent the host and the environment (Gordon 1950) O viously the first two are essential and since the environment may affe both the host and the infectious agent particularly in the transmission the latter from host to host it is the interplay between these three facto that determines the mass behavior of the disease If transfer of the infe tion to man or to some other vertebrate host is dependent upon an inse vector then the biologic environment in this case the insect vector becom equally as essential as the infectious agent and the vertebrate host Con quently a disease or at least its spread is not dependent on a single facto but rather on a combination of factors—the infectious agent the host an the biologic environment—each of which is obligatory

The epidemiologic pattern displayed is thus dependent upon the summ tion effect of all these factors If they were to remain static and not fluctua a phenomenon that is rarely if ever encountered in nature the mass b havior of the disease would be fixed and immutable The greater the var tion in these contributing factors the more varied is the epidemiology the disease and the factor that is subject to the greatest fluctuation tends dominate and determine the epidemiologic pattern It is with these conside ations in mind that the epidemiology of yellow fever is presented

The accumulated knowledge of the nature characteristics and poten alities of yellow fever virus the susceptibility of vertebrates to infection th symptomatology pathology and immunity produced in them by the viru

the ability of certain mosquitoes to transmit the infection as well as information on the ecology of these potential hosts and vectors has given in preceding chapters It is the purpose here to interpret and piece information together in an effort to understand as far as the known permit the epidemiology of the disease or better the natural history the virus

In order to avoid confusion an explanation of the terminology employed in this chapter is given here Man–mosquito cycle is used to designate the epidemiologic form of yellow fever resulting from the transfer of the virus from man to man through the medium of a mosquito vector It may occasionally be referred to as urban yellow fever Forest cycle or animal–mosquito cycle refers to the cycle of the virus occurring in forests in which man is not involved Jungle or sylvan yellow fever applies to the disease in man contracted from the bite of sylvan mosquitoes the virus being derived directly from the forest cycle Since the term yellow fever was originally used to designate the disease in man an effort will be made to restrict it to this meaning In referring to other hosts as well as vectors infection with yellow fever virus or some similar terminology will be employed

TRANSMISSION AND SURVIVAL OF AN OBLIGATE PARASITE

The virus of yellow fever like all viruses belongs to the category of obligate parasites therefore at this point the prerequisites for the transmission and survival of infectious agents of this nature will be considered briefly As implied by the name the active life and propagation of such parasites in nature is limited to the host in which they temporarily or more permanently reside There is no free living or reproductive phase beyond the host and the survival of the parasite is dependent upon the transfer from one susceptible host to another The speed of the transfer from host to host in the struggle of the parasite for survival depends upon the length of time it can remain in any given host and how long it may survive when separated from the host If it can remain alive and in a condition favorable for transmission a long period in a given host the transfer to another host is not urgent if the sojourn in the one host is brief the need for transfer to a second is pressing Likewise the urgency of reaching a second host after having separated from the preceding one will depend upon the length of time

the parasite is able to remain viable under the extraneous environment encountered.

It is of course essential that the parasite find a means of escaping from the host it is occupying in order to arrive at the succeeding host. It must be excreted or in some way made accessible for transfer, otherwise it is trapped and what has been termed a dead end infection results. The escape may take place through a number of channels, very commonly through the dejecta and excretions from the host. Also it may take place by means of an intermediary host frequently designated as a vector. Most active vectors are hematophagous and transmit parasites that circulate in the blood of the vertebrate hosts. Since the parasite multiplies in the bodies of these active vectors, they are as much hosts of the parasite as are the vertebrate hosts. It is also obvious that the parasite, after escaping from one host, must find a route of entrance into the tissues of the next host in line. This again may take place by several routes, including the intermediate host or vector.

Finally comes the role of environment, both physical and biologic. In the passive transfer of a parasite by water, food, and the like, it is the physical environment that is important. In transmission by direct contact, and certainly by an insect vector, biologic environment is the determining factor.

One other consideration warrants emphasis. If a number of different hosts or vectors are involved, or if the infection may be communicated by several different means, the epidemiologist should endeavor to assess the relative importance of each. This is essential for understanding the natural history of the parasite and for instituting methods for the control of the infection it causes. Too frequently it is the bizarre and the exceptional event that attracts attention, while the commonplace escapes notice or is not adequately appreciated. Yet it is the commonplace upon which the survival of the parasite depends, and if this is recognized and effectively attacked, the unusual may be disregarded.

These general principles are elementary and self-evident, but they should be borne in mind in rationalizing the information relative to the transmission and survival of the virus of yellow fever.

CHARACTERISTICS OF YELLOW FEVER VIRUS OF EPIDEMIOLOGIC SIGNIFICANCE

The virus of yellow fever multiplies only within or in close proximity to living cells. It has no extrahost life and at usual ambient temperatures will

survive for only a relatively short period outside the host. The host r
both in vertebrates and in arthropods is limited. In suitable vertebrate h
it circulates in high concentration in the blood but only for a brief per
rarely exceeding one week. A vertebrate host in which it produces infect
either dies or develops a staunch and persistent immunity. No chronic or c
rier stage of yellow fever in vertebrates has ever been observed. Thus t
virus must be transferred to another susceptible host during this brief perio
of active infection or it perishes.

If blood containing an adequate quantity of virus is imbibed by a suit
able mosquito host, the virus proceeds to multiply in the mosquito and
eventually reaches its salivary glands. Subsequently, when the mosquito
feeds upon a susceptible vertebrate host, the virus is injected into the skin
of the animal as a part of the salivary secretion and infection is initiated.
In contrast to its short duration in the vertebrate host, the virus persists
in the mosquito, and the insect may be capable of transmitting the infec
tion throughout the remainder of its life. The virus does not seem to have
any harmful effect on the mosquito or to influence the longevity of the mos
quito. However, no transovarian transmission has been revealed, and to
become infected the mosquito must imbibe infected material (blood from
an infected vertebrate).

In passing it may be mentioned that mosquito larvae may be infected by
exposing them to a liquid medium impregnated with the virus and that the
imagoes that emerge retain the virus. This observation is of experimental
interest but probably has no bearing upon the natural survival of the virus
(Whitman, 1943).

The virus has the ability of readily penetrating the slightly scarified skin
or even the undamaged skin (Bauer and Hudson, 1928b) and the mucous
membranes and stomach mucosa (Findlay and Clarke, 1935a, and Findlay
and MacCallum, 1939b) of a susceptible animal and producing infection.
This property of the virus has been used to advantage in inducing immunity
with attenuated strains and seemingly accounts for many of the laboratory
infections that have occurred. But for this means of transmission to occur
under natural conditions, it must be presupposed that the virus is able to
escape from a previously infected animal. Experimental evidence on the
excretion of the virus from an infected host is rather meager but such as
is casts doubt on the elimination of the virus through the feces, urine,
or vomitus. As has been noted, however, the blood is highly infectious, and
material containing unaltered blood would probably contain the virus.

It should also be recalled that the antigenic integrity of yellow fe[ver] virus has been well preserved and in this respect the virus differs from so[me] of the other viruses such as that of influenza. All the strains of yello[w] fever virus that have been isolated both in Africa and South America fro[m] forest mosquitoes and primates as well as from man are antigenically ver[y] similar if not identical. The question of natural variations in pathogenicity, tropism and host selectivity is more difficult to answer. Certainly these qualities may be profoundly modified by laboratory manipulation to wit the 17D and French neurotropic strains. There is in addition some evidence that infectiousness to certain forest animals (marsupials) (Waddell and Taylor 1948) may be enhanced by repeated cyclic passage of the virus in the species. Also some differences have been noted in the pathogenicity to primates and infectiousness to marsupials in strains that presumably have been altered little if any by laboratory handling (Laemmert 1944 and Anderson and Roca Garcia 1947). The same might be said of the relation of the virus to the mosquito vectors. As a result of the modifications induced in the 17D and French neurotropic strains it is very difficult if not impossible to transmit these strains by means of the mosquito. Viruses isolated in nature have not been adequately studied in this respect but it is possible that there may exist differences in the ease with which different species of mosquitoes are infected by them. Thus the practical import of strain variations must be left in doubt as it has not been experimentally proved that the magnitude of these differences is sufficient to affect the course of the cyclic passages of the natural strains of viruses or to modify the epidemiology of the disease.

EPIDEMIOLOGIC CONCEPT OF A HOST

In yellow fever parlance the term host is usually applied to the vertebrate and the term vector to the arthropod involved in the cycle. This nomenclature will be adhered to although as will be discussed later there is some question of its justification. In the experimental or laboratory sense an animal has been defined as being susceptible if it will permit the unequivocal multiplication of yellow fever virus within its body. To this extent it may be regarded as serving as a host to the virus but when applied to the epidemiology of the infection the meaning of susceptibility must be further qualified. It should be asked: Is the animal susceptible to infection under the conditions prevailing in nature? Can the animal transmit the in-

survive for only a relatively short period outside the host The host range both in vertebrates and in arthropods is limited In suitable vertebrate hosts it circulates in high concentration in the blood but only for a brief period rarely exceeding one week A vertebrate host in which it produces infection either dies or develops a staunch and persistent immunity No chronic or carrier stage of yellow fever in vertebrates has ever been observed Thus the virus must be transferred to another susceptible host during this brief period of active infection or it perishes

If blood containing an adequate quantity of virus is imbibed by a suitable mosquito host the virus proceeds to multiply in the mosquito and eventually reaches its salivary glands Subsequently when the mosquito feeds upon a susceptible vertebrate host the virus is injected into the skin of the animal as a part of the salivary secretion and infection is initiated In contrast to its short duration in the vertebrate host the virus persists in the mosquito and the insect may be capable of transmitting the infection throughout the remainder of its life The virus does not seem to have any harmful effect on the mosquito or to influence the longevity of the mosquito However no transovarian transmission has been revealed and to become infected the mosquito must imbibe infected material (blood from an infected vertebrate)

In passing it may be mentioned that mosquito larvae may be infected by exposing them to a liquid medium impregnated with the virus and that the imagoes that emerge retain the virus This observation is of experimental interest but probably has no bearing upon the natural survival of the virus (Whitman 1943)

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It should also be recalled that the antigenic integrity of yellow fever virus has been well preserved and in this respect the virus differs from some of the other viruses such as that of influenza All the strains of yellow fever virus that have been isolated both in Africa and South America from forest mosquitoes and primates as well as from man are antigenically very similar if not identical The question of natural variations in pathogenicity tropism and host selectivity is more difficult to answer Certainly these qualities may be profoundly modified by laboratory manipulation to wit the 17D and French neurotropic strains There is in addition some evidence that infectiousness to certain forest animals (marsupials) (Waddell and Taylor 1948) may be enhanced by repeated cyclic passage of the virus in the species Also some differences have been noted in the pathogenicity to primates and infectiousness to marsupials in strains that presumably have been altered little if any by laboratory handling (Laemmert 1944 and Anderson and Roca García 1947) The same might be said of the relation of the virus to the mosquito vectors As a result of the modifications induced in the 17D and French neurotropic strains it is very difficult if not impossible to transmit these strains by means of the mosquito Viruses isolated in nature have not been adequately studied in this respect but it is possible that there may exist differences in the ease with which different species of mosquitoes are infected by them Thus the practical import of strain variations must be left in doubt as it has not been experimentally proved that the magnitude of these differences is sufficient to affect the course of the cyclic passages of the natural strains of viruses or to modify the epidemiology of the disease

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In yellow fever parlance the term host is usually applied to the vertebrate and the term vector to the arthropod involved in the cycle This nomenclature will be adhered to although as will be discussed later there is some question of its justification In the experimental or laboratory sense an animal has been defined as being susceptible if it will permit the unequivocal multiplication of yellow fever virus within its body To this extent it may be regarded as serving as a host to the virus but when applied to the epidemiology of the infection the meaning of susceptibility must be further qualified It should be asked Is the animal susceptible to infection under the conditions prevailing in nature Can the animal transmit the in

fection by the means believed to apply in nature? Do the habitat and the ecology of the animal permit it to play a part in the epidemiology of the disease? According to these criteria hosts may be divided into three categories first those that may be infected but that cannot serve as a source of the virus for further transmission (dead end hosts) second those that may be infected and that may serve as a source for transmission of the virus by the means provided in nature but whose habitat does not normally bring them in contact with the virus (potential hosts) third those that may be infected and be a source of the virus under the proper biologic environment and whose habitat and ecology tend to bring them in contact with this environment (true or natural hosts)

Obviously main interest centers about the natural hosts i e the animals that play an integral part in the chain of events that is responsible for the survival spread and natural history of the virus While the concept of this type of host is simple it is by no means easy to secure reliable information for recognizing such hosts and especially for assessing the respective roles that each host species may play This requires a combination of tedious laboratory experimentation and a careful evaluation of field observations In a discussion of this problem Laemmert de Castro Ferreira and Taylor (1946) said

> In interpreting the practical implications of the experimental work on vertebrate hosts and arthropod vectors of the virus consideration must be given to the methods and criteria adopted in determining the susceptibility of a vertebrate and the ability of an arthropod to transmit the virus Also the knowledge gained in the laboratory should be weighed in the light of field experience and the ecology of the host and vector involved The one may serve to complement or to contradict the other

As may be expected the behavior of the virus and the response of the host are influenced by the dose of the virus employed and the route and manner by which it is introduced It is fair to assume from the evidence at hand that natural infection of animals probably results from introduction of the virus through the epidermis by the bite of an infected insect If this be true the experimental infection of animals by the intracerebral intraperitoneal or intramuscular route has no counterpart in nature and tests for susceptibility based upon such techniques may be questioned as far as the epidemiology of the disease is concerned On the basis of laboratory experiments only animals that may be infected by inoculation of the virus intradermally or subcutaneously or preferably by the bite of an infected insect

should be regarded as potential hosts. It should also be borne in mind that an animal can play a role in cyclic transmission of the disease only when the virus, following its introduction, multiplies to such an extent as to attain a concentration in the blood sufficient to infect the insect vectors. It has been found, for example, that some species of mammals following exposure to infected mosquitoes may circulate virus in low concentration and subsequently develop neutralizing antibodies and yet be unable, or only rarely able, to pass the infection on to normal mosquitoes. It is not improbable that such low grade infections giving rise to circulating antibodies may occur in nature when mosquitoes carrying the virus are present. Distinction should be made, therefore, between these low grade, or so-called "dead end," infections and those in which the virus circulates in adequate concentration to be readily transmissible to an insect vector, since the former can play no part in the continuous propagation of the virus in nature. It is obvious that the best laboratory means of ascertaining whether an animal may be integrally involved in the epidemiology of the disease is to determine if the virus may be maintained for a series of cycles by alternate passage through the animal and a known efficient vector. Indeed, in its epidemiological application it would be desirable to limit the term susceptibility to vertebrates that satisfy this criterion.

Comparison and evaluation of reported laboratory experiments are further complicated by differences in host affinity among some of the jungle strains of the virus. It appears to be fairly well established that whereas certain mammalian species are rather easily infected by one strain of jungle virus, they are resistant to infection with another strain. Lastly, it has been observed that the neutralization test, while highly specific in man and, with few exceptions, among the primates, is not so dependable in other mammalian orders. Apparently some of the marsupials and especially certain species of rodents possess nonspecific virucidal substances in the blood capable of inactivating yellow fever as well as other neurotropic viruses. This phenomenon tends to vitiate the results of immunity surveys in these animals. The above remarks will serve to indicate that laboratory experiments on suspected hosts should be viewed critically and their epidemiological implications interpreted with caution.

EPIDEMIOLOGIC CONCEPT OF A VECTOR

What has been said in regard to the epidemiologic concept of a vertebrate host also generally applies to the vector. This agent must be subject to infection under natural conditions; it must be able to transmit the in-

lection to a susceptible host and its range of habitat must bring it in contact with the virus and with suitable vertebrate hosts

Since it is presumably only by bite that an arthropod can acquire or transmit the virus in nature this is taken as the basis of determining the ability of an arthropod to serve as a vector With this in mind and with due consideration given to ecology arthropods like the vertebrates may be divided into three classes those that may be infected but are unable to *transmit the virus to a susceptible vertebrate (dead end vectors)* *those that* may be infected and may transmit the virus to a susceptible host but whose natural habitat is not such as to bring them in contact with the virus and with susceptible vertebrate hosts (potential vectors) and those that may be infected and may transmit the virus to a susceptible host and whose normal habitat brings them in contact with both the virus and susceptible hosts (true or natural vectors)

The first group dead end vectors are not really vectors *in the strict sense* of the word as members of this group are not able to pass on the virus This classification is made to distinguish them from arthropods that are able neither to harbor nor to transmit the virus

The question has been raised as to the propriety of applying the term vector to arthropods and the term host to vertebrates Viewed from the *aspect of the disease or the infection in man* and other vertebrates this nomenclature is quite correct as it is the arthropod that plays the vectorial role From the aspect of the time element and the reservoir of the virus however the mosquito qualifies better as a host than does the vertebrate for it is in the mosquito that the virus finds a more permanent abode In the vertebrate its life is fleeting and it must be rescued by the mosquito within a few days in order to survive However by common usage the application of these terms has been well established and no change is now proposed but *in contemplating the cyclic life of the virus it is well to remember* the above mentioned facts

INFLUENCE OF ENVIRONMENT

The role of the third factor environment will become evident in describing the epidemiologic behavior of yellow fever virus but it may be permissible at this juncture to make a few generalizations For descriptive purposes *environment is usually divided into three categories*—physical biologic and socioeconomic The divisions are obviously artificial as they

are co-related and one type of environment may drastically affect the other. This is evidenced especially by physical environment which is the dominant influence upon biologic environment and may also determine to some extent the socioeconomic environment. It is of interest, however, to examine first the direct effect of each of these environmental categories.

Physical environment per se would seem to have only a limited effect upon the propagation of the virus except that low temperatures curtail its multiplication within the mosquito. It apparently has no influence upon the course of the disease in warm blooded animals. The erection of physical barriers such as screening between the host and the vector and the existence of suitable breeding places for the latter are in reality indirect effects acting through the medium of biologic environment.

The same may be said of *socioeconomic environment*. It is of importance only in exposing persons to the infected vector through occupation or through the place and character of residence.

On the other hand, the biologic environment viewed from the aspect of the virus is all important. It is, indeed, the biologic environment—hosts and vectors—that sets the course and determines the epidemiologic pattern of the disease, or, it would be better to say, the natural history of the virus. The biologic environment is in turn dependent upon the physical environment, and for this reason yellow fever is limited to areas and seasons of the year in which the climatic conditions support the presence of the required hosts and vectors in sufficient numbers. The ecology of these hosts and vectors and the gross and microclimatic conditions they demand will be discussed later in reference to the geographic regions harboring the virus of yellow fever.

LABORATORY ADJUNCTS TO EPIDEMIOLOGIC INVESTIGATIONS

In few other instances has laboratory research been so fruitful in developing techniques applicable to epidemiologic studies as it has been in the case of yellow fever. Without these laboratory aids many of the now recognized phases of behavior of the virus in nature would still be obscure. With them it has been possible to clarify events that previously were either inexplicable or overlooked. These laboratory procedures have been referred to and described in other chapters, and it remains only to indicate their use in epidemiologic investigations.

PROTECTION OR VIRUS NEUTRALIZATION TEST

Fortunately for the purpose of tracing yellow fever virus infection hrough vertebrate hosts the infection leaves behind it an immune trail n man and other primates the immunity is permanent with few exceptions ighly specific and is detectable by the neutralization test As a means of etrospective diagnosis this test has proved invaluable It has permitted hrough serologic surveys the delineation of the areas where yellow fever has occurred within the life of the human population and of those in which he existing monkeys have been infected with the virus Used in periodic urveys it shows whether or not yellow fever virus infection has occurred n a human or monkey population during the intervals between the surveys

Its application to other species of animals though perhaps not so con lusive as with the primates has revealed suggestive information Sera of everal species of the marsupials will rather consistently give a positive neu ralization reaction following infection with the virus but the immune re ponse in these animals may not be so dependable or permanent as in mon eys and man The specificity of the test particularly in rodents as well as n a number of other orders of mammals has been questioned and caution hould be exercised in interpreting positive reactions in animals other than primates Nevertheless the test has been of great service in obtaining either lependable or presumptive data on vertebrates naturally infected with the irus

COMPLEMENT FIXATION REACTION

While this reaction has been the subject of considerable study by various bservers it has not assumed such an important place in the diagnosis of ellow fever as the neutralization test If properly performed it appears to be reasonably reliable in both man and monkeys but in time of appearance s well as in persistence it is not as consistent as the virus neutralization eaction The fact that it may disappear within a limited period in both nan and monkeys makes it of some use in conjunction with the neutraliza ion test in estimating the time when the infection occurred that is a serum howing both complement fixing and neutralizing antibodies indicates a nore recent infection than a serum showing neutralizing antibodies but no complement fixing antibodies However the persistence of the comple nent fixing antibodies is quite variable and differs with different species of animals as well as with individuals

It may also be added that the test is not applicable to certain species of animals For example some species of marsupials react to infection by the production of neutralizing antibodies but fail to develop complement fixing antibodies

HISTOPATHOLOGIC DIAGNOSIS

In a previous chapter the liver lesions produced in man by yellow fever have been described These as a rule are characteristic and will allow an experienced pathologist to diagnose the disease with considerable accuracy This rather characteristic pathologic picture has been used to advantage in the recognition of fatal cases To facilitate the securing of liver specimens a viscerotome was devised With this simple instrument a satisfactory specimen of liver can be secured from a cadaver by a layman after brief instruction The procedure causes no mutilation of the body and is therefore not objectionable to the family Through the establishment of viscerotomy posts for securing specimens of liver from persons dying of acute febrile illness the recognition of yellow fever has been greatly augmented In practice these viscerotomy services which now prevail in all South American countries where yellow fever is endemic have proved to be the most valuable means of identifying hidden foci and epidemic excursions of the disease

ISOLATION OF VIRUS

The isolations of virus from captured vertebrates and arthropods have not been abundant but such isolations have materially helped to clinch the validity of neutralization tests and to confirm epidemiologic evidence As the virus circulates in mammals for only a very limited period the chances of capturing an animal while in the active stage of infection are obviously rather remote On the other hand during human epidemics there is usually no difficulty in obtaining the virus from man if the blood is taken during the acute phase of the disease In this instance the onset of suspicious symptoms serves to indicate when the blood should be taken Because the mosquito harbors the virus for a longer period the opportunities of obtaining the virus from this source are vastly greater The virus has been isolated much more frequently from mosquitoes than from captured primates Indeed it was only during one epizootic in South America that the virus was isolated from wild marmosets It has however been obtained in Africa on several occasions from sentinel monkeys By taking daily tem

peratures of these sentinel monkeys acute infection with yellow fever ha been recognized and by securing blood during the febrile period succes in isolating the virus has been achieved While the virus has been obtaine on numerous occasions from mosquitoes it is rather surprising that it ha not been isolated more frequently in forests presumably harboring the viru and particularly from urban mosquitoes during human epidemics

EPIDEMIOLOGIC BEHAVIOR OF YELLOW FEVER VIRUS

In the foregoing pages the products of laboratory research on the virus and its hosts and vectors have been summarized There remains the task of correlating and rationalizing this knowledge with field observations

Two main patterns of yellow fever virus infection are now recognized the man–mosquito cycle and the forest or lower animal–mosquito cycle and as a by product of the latter jungle yellow fever acquired by man through the bite of infected sylvan mosquitoes The virus involved in each is essentially the same and may be transplanted from one cycle to the other It is as mentioned before the biologic environment or the kind of hosts and vectors available that regulates the course the virus will follow It is known that man is frequently infected by forest contact and that the virus derived from the forest may initiate the man–mosquito cycle and though difficult to prove it is not unlikely that the converse may occasionally occur

It will be convenient however to treat these two cycles separately and then show how they may be integrated It will also be convenient to consider each cycle according to regions e g as it occurs in the Americas and as it occurs in the Old World principally in Africa

MAN–MOSQUITO CYCLE

This is the simplest and best known cycle It involves only one host—man—and usually in any given area only one species of mosquito With possibly rare exceptions the vector from man to man is a domestic mosquito that breeds and dwells about human habitation and the female of which has a predilection for human blood It therefore lends itself better to bionomic study than do forest mosquitoes Likewise yellow fever in man is more easily recognized and followed than is the disease in forest animals

The elements involved in the spread and persistence of the infection

after it is introduced into a community are fundamentally the density and dispersion of the vectors and the size and the stability of the susceptible human population. Other factors of consideration are the incubation period of the disease in man (3 to 6 days), the period of circulation of the virus in the blood of man (not exceeding 4 to 5 days), the length of time required before the mosquito can transmit the virus after taking an infective blood meal (8 to 12 days), and the longevity of the mosquito after it has become infectious (6 to 8 weeks). These time estimates are approximate but probably represent the usual limits. In exceptional instances they may be somewhat shortened or prolonged. The data on mosquitoes refer to observations on epidemics transmitted by *Aedes aegypti*. Carter (1900 and 1901*a*) noted that the interval between the occurrence of the primary and secondary case averaged about two weeks, with a minimum of 11½ days. Allowing for an incubation period in man of 3 to 6 days (Carter, 1901*b*), this would place the usual interval required for the mosquito to become infectious at about ten days. Carter has termed this the extrinsic incubation period.

Because a proper balance between the susceptible hosts and vectors is required for continuation of the cycle, it is obvious that events tending to upset this balance, such as natural or induced measures that critically diminish the number of vectors or limit the number of susceptible hosts, will alter the mass design of the disease. The limitation of hosts is conditioned largely by the size and the stability of the community involved and the existence and development of immunity. Lastly, the area spread of the infection is determined by the continuity and movement of the human population and the flight range and artificial transport of the infected vector.

MAN—MOSQUITO CYCLE IN THE AMERICAS

This cycle exemplifies the classic yellow fever which, with periodic exacerbations and recessions, was a scourge to tropical and subtropical ports for more than two hundred years. On occasions the epidemics extended inland, principally along water routes, and made excursions as far north as Boston and southward to Buenos Aires. For a presentation of the evidence on the origin and early history of the disease, reference is made to Carter (1931). The present brief review will be confined to events since the turn of the last century, when transmission of the infection by the *A. aegypti* mosquito was unequivocally proved. From a practical aspect this

species of mosquito is the sole vector of the disease from one human being to another in the Americas though as will be referred to later in connection with the forest cycle sylvan mosquitoes have been suspected of occasionally transmitting the virus among members of the same families living in close proximity to forests or among workers on coffee plantations but here *A aegypti*–transmitted yellow fever is the only concern

Long before 1900 the disease was seeded well along the east and west coasts of South America and about the Caribbean Campaigns against the mosquito vector were successful in suppressing the disease in Havana Vera cruz the Isthmus of Panama Rio de Janeiro and likewise in New Orleans in 1905 where yellow fever made its last appearance within the borders of the United States Similar campaigns from 1910 to 1913 were effective in arresting urban infections along the Amazon in Pará Manaus and Iquitos In 1919 and 1921 yellow fever was eradicated from the west coasts of Ecuador and Peru and has never reappeared on the western seaboard of South America There were outbreaks in Guatemala El Salvador Nicaragua Honduras and British Honduras from 1918 to 1921 and again in El Salva dor in 1924 In Mexico the disease was unusually active in 1920 on both the eastern and western coasts and epidemics occurred in the cities of Vera cruz and Tampico but since 1922 no yellow fever has been recorded in Mexico In 1923 there was a limited outbreak in the interior of Colombia at Bucaramanga By 1925 the disease appeared to be confined to a region in northeastern Brazil and it was hoped that it was on the point of being eradicated from the New World but in the following year owing to move ment of nonimmune troops through the region there was a sudden increase in the cases reported In 1928 and 1929 there was a more serious develop ment yellow fever again appeared in Rio de Janeiro after an absence of more than 20 years and in spite of a vigorous antimosquito campaign there were 435 deaths before the epidemic was brought to a halt To date this has been the last epidemic in the Americas involving a large city (For original references see Sawyer 1932 and Soper 1938*a*) However there was a small epidemic in the town of Socorro in Colombia in 1929 (Pena Chavarría Serpa and Bevier 1930) and for several years *A aegypti*–transmitted yellow fever continued to exist in northeastern Brazil But the elimination of the infection in the latter region in 1934 sounded the death knell of the sus tained man–mosquito cycle of yellow fever virus in the Americas (Soper 1938*b*) It should not be forgotten however that the virus is wide flung in

the tropical forests of South America and if introduced into urban a
where *A aegypti* exists may give rise to epidemics

Although previously mentioned by Soper (1936*a*) the first detailed
count of the forest virus coming to town and initiating an urban epide
was made by Walcott Cruz et al (1937) *In retrospect it is now reason*
certain that many if not all of the inland urban outbreaks in regions wh
there are neighboring forests which suddenly appeared after long inte
of silence and without any connection with other urban foci may be
tributed to introduction of the forest virus Such an event probably expl
the outbreaks in Colombia at Bucaramanga in 1923 at Socorro in 1
in Bolivia at Santa Cruz de la Sierra in 1932 and probably many other
the past Limited epidemics of this nature were discovered in Brazi
1935 at Theophilo Ottoni State of Minas Geraes in 1936 at Lábrea S
of Amazonas at Figueira State of Minas Geraes and at Cambara Stat
Paraná in 1937 at Xapurí Territory of Acre and the last in 1942 at S
Madureira Territory of Acre In Bolivia according to Torres Muñoz
last yellow fever to occur in the presence of *A aegypti* was at Terel
near Santa Cruz in 1936 and in Peru according to Lazarte the last occu
at Contamana in 1937 and Tarapoto in 1942 It was reported by Gast G
from Colombia that none of the liver specimens found positive for ye
fever since the establishment of the Viscerotomy Service in 1934 came f
localities where *A aegypti* was present

The fundamental facts governing the epidemiology of urban yellow f
have been well presented by Carter in his book on the early histor
yellow fever (1931) It is therefore necessary to give only limited spac
the discussion of this epidemiologic form of the disease beyond indica
what has been subsequently contributed as the result of the developmen
laboratory methods applicable to epidemiologic studies

BIONOMICS OF A AEGYPTI IN THE AMERICAS

As the epidemiology of the man—mosquito cycle is governed largel
the vector some of the characteristics and habits of this mosquito
have a direct bearing upon its vector potentialities will be reviewed
rather generally agreed among entomologists that the species *A aeg*
probably originated in Africa and at some time in the past was transpla
to the New World Yet the American strain differs in some of its habits f
its African forebears particularly in respect to its domesticity Its ha

is limited to the vicinity of human dwellings, it breeds almost exclusively in artificial collections of water about houses, and it feeds largely upon man preferably by day, but also by night The imago tends to remain near its breeding place and its effective overland flight range is not great, probably within 300 m, but it may under force of circumstance fly somewhat greater distances, as, for example, when released from an off shore boat (Shannon Burke and Davis 1930, and Shannon and Davis 1930) This latter observation is of considerable importance in connection with the transport of the mosquito from one seaport to another and along river routes

It is a prolific breeder, and under favorable conditions the complete cycle from egg to imago may take place within 10 days It is in all stages, including egg, larva, and adult, rather sensitive to extreme temperatures The *adult becomes inactive at a temperature below 15°C and is rapidly killed* when 0°C is approached The limits for functional activity—breeding and feeding—are usually given from 68° to 102 5°F (Carter, 1931) and the egg will not survive for more than a few days at temperatures much below freezing (N C Davis 1932*b*) This sensitivity to low temperatures is a limiting factor in its geographic distribution As Howard Dyar, and Knab (1912) have stated, its permanent distribution is determined by the minimum temperatures and its temporary distribution by the maximum temperatures of any given region wherever it is sufficiently populated" Consequently for its permanent survival, the temperature must not be so low in winter as to destroy the eggs and must be sufficiently high in the summer to favor reproduction For these reasons the mosquito is confined in a permanent state to tropical and the lower austral isotherms Carter set Norfolk, Virginia, as about the northern limit for *A aegypti* survival at sea level on the Atlantic coast Below the equator the zone of permanent survival is somewhere between southern Brazil and Buenos Aires, and on the west coast to or somewhat beyond the southern boundary of Peru

Yellow fever can persist in endemic form only in climates where the imago remains alive throughout the year, as it is only the imago that acquires and transmits the virus There are borderline areas in which the imagoes die out during the winter season but eggs may survive and mosquitoes thus reappear with the warm season In these instances only the introduction of the virus is required to initiate an epidemic Such an event may have occurred on one or two occasions in Philadelphia In unusually mild winters infected imagoes may survive and with the return of warm weather initiate

epidemic without further introduction of the virus It was suggested by rter (1931) that this may have happened in New Orleans from 1897 to)8

Information on the longevity of the mosquito under natural conditions is ficult to acquire Shannon Burke and Davis (1930) were able to capture rked aegypti only up to 17 days after release However the mosquito has n kept alive in captivity for a much longer period Perhaps the best evi nce of the life span from an epidemiologic point of view is that it re ires from 6 to 8 weeks to suppress yellow fever completely after the breed , of the mosquito has been arrested by control measures (Carter 1922b nnor and Monroe 1923)

As the life and existence of this mosquito in the Americas is closely linked that of man and as its flight range is rather limited its dissemination to stant parts is dependent upon human intercession Consequently it has llowed the main routes of travel and commerce In the early days of ocean avel by sailing vessels when fresh water was carried aboard ship in open ntainers that furnished favorable breeding places for the mosquito it as by this means transported from port to port It was also transported by land river commerce and to a lesser degree by railroads and overland agon travel As a result its distribution was limited mainly to seaports aterways and other frequently traveled routes It therefore never was dis rsed widely among small inland communities and isolated farmhouses en in regions where climatic conditions were favorable for its propagation owever in areas of scanty rainfall where drinking water is at a premium d frequently is carried from place to place by travelers eggs or larvae mosquitoes may be carried in this way to small communities or even olated habitations Such a situation was observed in northeastern Brazil

THE HOST

As far as is known man is naturally more or less uniformly susceptible to fection with yellow fever virus with the possible exception of infants born f immune mothers such infants may be immune for the first few months of eir lives However there is marked variation in the severity of the disease n the native African Negro the disease appears to be milder than in other aces (Carter 1931 Beeuwkes 1936) This may be due to genetic selection esulting from long racial exposure to the virus but a wide range in the everity of the attack is commonly noted in epidemics irrespective of the

racial stock involved No convincing evidence has been presented that the physiologic state as it may be influenced by nutrition physical exposure and the like exerts an effect upon susceptibility to infection or upon the *subsequent course of the disease Nor if one may judge* from experimental infection of rhesus monkeys does the size of the infecting dose of the virus appear to influence markedly the outcome of the resulting infection

While the severity of the disease in man is of great human importance it probably has little influence upon the spread of the infection The virus circulates in the blood stream even in those having very mild attacks and presumably in sufficient concentration to infect mosquitoes Persons who recover are eliminated as sources of the virus as rapidly as those who die because of the very short period during which the virus is present in the blood stream

Since an attack of the disease creates a firm resistance to subsequent infection the immunes increase and the susceptibles diminish as the epidemic progresses This event combined with the size of the original susceptible population involved and the replacement of nonimmunes by birth and by the influx of nonimmunes from the outside is the main contributing factor to the mass behavior of yellow fever as far as the human host is concerned

THE DISSEMINATION OF THE VIRUS

It will again be recalled that no material antigenic differences have been observed in various strains of yellow fever virus Presumably all strains are infectious to man and strains derived from the forest cycle give rise to urban epidemics that are in no way different in their clinical pathologic and epidemiologic aspects from epidemics arising from strains that have been sustained for a long period by the man–mosquito cycle It is suspected however that there may be considerable variation in strain virulence Besides dissemination by direct extension within the flight range of the mosquito and by the local movements of man the virus may be transported and introduced into a new locality (*a*) in infected mosquitoes (*b*) in infected man or (*c*) in a combination of the two Subsequent events and their rapidity of development depend upon the manner of introduction of the virus and the biologic environment encountered in the new locality

If the virus is introduced in infected mosquitoes human cases may develop within the incubation period in man that is within 3 to 6 days If there are no aegypti present and if the climatic conditions are not suitable

for the breeding of this mosquito the epidemic will be limited to the persons directly infected by the transported mosquitoes. If on the other hand climatic conditions are suitable for the breeding of this mosquito and particularly if it is already present secondary cases resulting from infection of additional mosquitoes may be expected and the epidemic will be more prolonged and extensive.

If the virus is introduced in an infected man and aegypti are present secondary cases will not develop for a period of approximately 2 weeks as noted by Carter (1900).

The most rapid development of an epidemic without the delay in the appearance of yellow fever in the local population occasioned by the period of intrinsic incubation may be expected when the virus is introduced in a combination of both man and mosquitoes and when aegypti are already present in the community. In the event aegypti are not present in the community and the epidemic must be established by the mosquitoes introduced at the same time as the virus the development of the epidemic is more insidious and its spread is limited to the extension of the newly seeded vector.

In retrospect it may be presumed that the former epidemics in the temperate zone represented various combinations of these circumstances. For instance the limited epidemic that occurred once at Halifax and did not spread beyond those having immediate contact with a ship introducing the virus is an example of the transport of the virus by infected mosquitoes into an area where the vector did not exist and where climatic conditions were unsuitable for its establishment. In the extensive epidemics that occurred in Philadelphia and further south it seems probable that aegypti were present at the time the virus was introduced. Once the virus is introduced the mosquito and human population govern the behavior of the disease from then on. The interplay of these two elements was discussed by Carter (1931) as follows:

> Very obviously continuance of yellow fever in a community will lessen the number of people susceptible to that disease—each one attacked becoming immune to it at least temporarily and when this continuance has reached such a degree that no aegypti infective with yellow fever has access to the man susceptible to yellow fever the disease will cease. This the writer (Carter 1917) has called "the spontaneous elimination of yellow fever by failure of the human host" and it has been of quite common occurrence even where from temperature conditions and the customs of the people *aegypti* continue active during the whole year.

Obviously it is not necessary to exterminate *aegypti* in order to eliminate yellow fever from an infected place If the number of mosquitoes be brought below the critical number for yellow fever—an idea we owe to Ross as applied to malaria and extended to yellow fever by Gorgas (1908)—at that place the disease will die out Note too that this critical number for any place will vary directly as the proportion of men immune to yellow fever in the total population thus if with one hundred cases of yellow fever introduced into a community in which all were susceptible to yellow fever the number of *aegypti* were such that exactly one hundred men would be in fected from these cases the disease would neither die out nor increase This would be the critical number of *aegypti* for that place and time With less mosquitoes than this less than one hundred men would be infected and the fever would die out With more mosquitoes more than one hundred men would be infected and it would increase Now if one fourth of the inhabi tants were immune to yellow fever obviously the same number of mos quitoes which infected one hundred men before would now infect only seventy five—one fourth of their bites going to immunes and hence being wasted—and at this rate the disease would die out The number of mos quitoes required to infect the one hundred men and hence just perpetuate the fever would have to be increased by one third above the first number

Obviously then this critical number below which the *aegypti* must be brought to eliminate fever is less in a community as the proportion of sus ceptible people increases Obviously also the number of susceptible men in a community compatible with the elimination of yellow fever by the failure of the human host varies inversely more or less with the number of *aegypti* in flight

This reasoning led (*a*) to the development of the key center concept namely that yellow fever could remain endemic only in large centers of human population where there was a continuous adequate supply of sus ceptible persons furnished by the newborn or by the influx of nonimmunes and (*b*) to the theory that if yellow fever could be eliminated from such cen ters it would disappear since in small communities where these conditions were not met it would burn itself out by immunizing and eliminating the susceptible human hosts irrespective of any measures taken against the mosquito Thus it was conceived that by attacking these key centers and reducing the mosquito population to a sufficient degree to arrest transmis sion yellow fever could be eradicated from the New World This concept based on the then existing knowledge was fundamentally sound but it ig nored two facts that subsequently came to light The first and less impor

tant was that aegypti-transmitted yellow fever might exist indefinitely in areas other than the large cities. This possibility was recognized (Carter 1917 and 1931) in Yucatan and Venezuela and termed "hacienda endemicity." It was not realized what a serious problem this might be until so-called rural yellow fever was encountered in northeastern Brazil (Soper 1934). However, even this obstacle was not insurmountable, as the man—mosquito cycle of yellow fever in this region was arrested in 1934. But the deathblow to the concept of driving yellow fever out of the Americas through control of the man—mosquito cycle came when it was shown that the virus is retained in tropical forests by a cycle independent of man.

EPIDEMIC YELLOW FEVER

The epidemic form of yellow fever under natural conditions occurs in regions not favorable for the year-round survival of aegypti mosquitoes and also where the human population involved is small and relatively stable. In the first instance the disease is terminated by the failure of the vector and in the second by the development of sufficient immunity in the human population.

Adequate information is not at hand to evaluate statistically the exact numbers of mosquitoes required to transmit the virus continuously in a susceptible population. Mosquito indices have been devised, and it has been found in many instances that when the index falls below certain levels yellow fever transmission ceases, but the reduction in mosquitoes required to arrest transmission would naturally vary with the percentage of susceptibles in the human population, which was not determined.

The data on the immunity rate necessary to terminate an epidemic in the presence of an adequate number and distribution of mosquitoes are likewise insufficient, primarily because an immunity test was not developed until long after mosquito control was being applied actively. For this reason no recognized epidemics have been permitted to run their natural course, and thus the number of mosquitoes and the ratio of immune to susceptible individuals have not been ascertained throughout an epidemic. It may be mentioned, however, that retrospective serologic surveys conducted in Brazil have revealed an immunity ratio in the age group under 15 years as high as 90 per cent (Soper 1937*a*). Perhaps the best example of such a survey conducted shortly following a recognized epidemic was one made at Cambucy, Brazil, which showed immunity, according to the mouse protection

test of 51 5 per cent among persons under 20 years of age (Soper a
Andrade 1933)

ENDEMIC YELLOW FEVER

The endemic form of aegypti transmitted yellow fever is by virtue o dependence on the mosquito s remaining active throughout all season the year confined to tropical and subtropical climates It has been clas into two categories urban and rural Endemic urban yellow fever occ only in rather large cities where the permanent or transient population sufficiently great to support its continuation

Endemic rural yellow fever demands rather special circumstances whic in brief are low rainfall necessitating the collection and storage of water in artificial containers a sufficient number of nonimmune travelers and a wide dissemination of the mosquito vector among the villages and isolated habitations

On the basis of origin a third category may be added namely yellow fever initiated by the forest virus This usually occurs in towns and villages situated near tropical forests harboring the virus Presumably the virus is introduced by a person who is infected in the forest and comes to town during the acute stage of the disease Obviously it is necessary for aegypti mosquitoes to be in the town at the time the virus is introduced

The advances in knowledge since the publication of Carter s book in 1931 are attributable largely to the application of methods of laboratory diagnosis which have permitted the recognition of yellow fever that otherwise would have escaped attention The changes wrought in the concept of the disease by the application of these methods in Brazil have been summarized by Soper (1934) as follows

1920	*1933*
Severe clinical disease considered typical	Severe classical case considered atypical in native population of endemic areas
Absence of reported cases indicating absence of disease	Absence of reported cases not accepted as absence of disease
ellow fever essentially urban and transmitted only by *A aegypti*	Yellow fever may continue at least for a period of months in rural areas with transmission by *A aegypti* or even in the absence of this mosquito
center control believed effective clearing surrounding area	Key center control not effective in Brazil

Epidemiology

Transmission of yellow fever in the absence of *A. aegypti* was design later as jungle yellow fever and will be treated separately. The stateme that key center control is not effective in Brazil should be modified if t term key center be broadened to include foci of endemic hacienda or rur yellow fever, for as a matter of fact continuous aegypti-transmitted yello fever was eliminated from Brazil the following year (1934).

The age and sex distribution of aegypti-conveyed yellow fever will be discussed later in conjunction with jungle yellow fever.

MAN—MOSQUITO CYCLE IN EUROPE

The epidemiology of the epidemics of yellow fever that invaded Europe in the latter part of the eighteenth century and during the nineteenth century was probably analogous to that of the excursions of the disease into the temperate zones of the Americas. It is presumed that these epidemics were transmitted by *A. aegypti*, and what has been said in regard to the former occurrence of yellow fever in the United States also applies to Europe. As a matter of fact, it is certain that many of these epidemics resulted from the introduction of the virus from the New World. It naturally follows that the course of the epidemics and their severity was related to the suitability of the climate to the propagation of the mosquito vector. In Europe, as in the United States, the disease did not become established endemically. Usually the winter temperatures were too low to permit the survival of the imago and the virus from year to year, although in some instances in southern Europe the virus may have been carried over from one summer season to the next as a result of mild winters.

MAN—MOSQUITO CYCLE IN AFRICA

Since we are concerned here only with the transmission of the disease from man to man and not with its origin and early history on the Dark Continent, reference will be limited to studies made subsequent to the discovery of the transmission of the virus by mosquitoes, as it was only after this fact came to light that any consideration was given to the mosquito fauna and its relation to yellow fever.

Though competent observers had concluded that yellow fever was en demic on the west coast of Africa (Boyce, 1910 and 1911; West Af ow Fever Commission, 1911) it was not until

munity tests were developed that it was proved to be identical with the disease diagnosed as yellow fever in the Americas While the man—mosquito cycle in parts of Africa is believed to be understood fairly well, there are certain inherent obstacles to following the behavior of the disease in the native population and to unequivocally determining the vector These obstacles are the scarcity of trained physicians, the almost complete lack of reliable birth and death reports combined with the habit of burying the dead under the house or within the compound, the resort of the natives to witch doctors when ill and the failure to consult European physicians even when they are available (Beeuwkes, 1936) Furthermore it is believed by some that the disease normally runs a milder course in Negroes and is thus more difficult to recognize In any event, clinical manifestations and mortality records are of limited avail in identifying endemic yellow fever or in early recognition of epidemics Moreover, it has not been feasible for administrative reasons and because of religious prejudices to obtain large numbers of liver specimens through the establishment of viscerotomy posts, the procedure that has been so successful in currently revealing silent foci of the infection in South America Only in the Belgian Congo has this method met with limited success When it comes to tracing the cycle in the mosquito there is the difficulty that not only one but a number of species of mosquito are experimentally capable of transmitting the virus which may be present in the outskirts or even in the midst of urban communities Finally the relative prevalence and to a significant degree the ecology of these mosquitoes vary in different regions of the continent, and what may apply in one region does not necessarily follow in another.

Notwithstanding the obstacles and complications encountered, much useful information has been accumulated on the distribution of the disease through immunity surveys the behavior of epidemic outbreaks, and the mosquito fauna associated with these epidemics *The following species of* African mosquitoes have been shown able to transmit the virus under experimental conditions *Taeniorhynchus africanus, A aegypti, Aedes africanus, Aedes luteocephalus, Aedes metallicus, Aedes simpsoni, Aedes vittatus, Aedes stokesi, Aedes taylori, Eretmapodites chrysogaster,* and *Culex thalassius*

In West Africa it seems unnecessary to postulate any other vector than *A aegypti* to account for the transmission of the disease in urban communities This conclusion rests upon the consistent presence of this mosquito in all towns and cities where epidemics of the disease have occurred and where

the immunity rate in the human population is high, the low immunity r in cities with piped water supplies where conditions are not favorable to intensive breeding of this mosquito, the peculiar breeding and feedi habits of A. aegypti which make it a better qualified vector of the infectio than any other of the species shown to be capable of transmitting the viru and the isolation of yellow fever virus from aegypti on four occasions, onc by Beeuwkes and Hayne (1931) and three times by Bugher and his co workers in 1946.

Kerr (1933) noted that in the region of Lagos, Nigeria, A. aegypti is the species that above all others prefers human blood and is found in greatest abundance in the vicinity of and particularly within human habitations. Bugher and his associates investigated an epidemic of yellow fever occurring at Ogbomosho, Nigeria, in 1946, and concluded that A. aegypti was the responsible vector. Thus all positive and circumstantial evidence points to A. aegypti as the principal if not the sole vector of yellow fever in West African towns and cities. However, the possibility that some other species such as A. africanus occasionally conveys the infection from man to man cannot be disregarded.

In the great rain forests of Central and East Africa another situation prevails. Though aegypti is widely distributed in these forested areas, it occurs in such small numbers, particularly about human habitations, that it cannot be seriously considered as a vector. On the other hand, A. simpsoni, a mosquito breeding in plant axils, is commonly abundant in villages and is regarded as the principal vector in the forest regions not only in transmitting the virus from monkeys to man but also in continuing the infection in the man–mosquito cycle once the virus is introduced (Mahaffy, Smithburn et al. 1942; Smithburn and Haddow, 1946). The habits of A. simpsoni fit into the epidemiologic picture of the human disease in Uganda, and the virus has been isolated from this mosquito on four occasions.

Investigation of an extensive epidemic in the Nuba Mountains, Anglo-Egyptian Sudan, in 1940 suggested still another transmission complex. At the time the epidemic was in progress no comprehensive entomologic studies were made, but Kirk (1941) implied that A. aegypti may have been the principal vector as it was rather widely distributed and quite prevalent in many of the villages involved, although it was not found in others. He suspected also that A. vittatus may have been concerned in the transmission of the virus. Lewis (1943) made an intensive entomologic survey the following year and came to the conclusions presented in Table 18.

TABLE 18

RELATION OF CERTAIN MOSQUITOES TO YELLOW FEVER IN THE NUBA MOUNTAINS ANGLO EGYPTIAN SUDAN

Species	*Estimated role*	*Notes on adults*
Aedes		
vittatus	Very important	Abundant on and near hills
furcifer *	Important	Numerous almost everywhere
taylori	Important	Numerous almost everywhere
luteocephalus	Of some importance on and near hills	Common on and near hills
metallicus	Important in some villages	Common near breeding places
aegypti	Important in some villages	Common near breeding places
simpsoni var *lilii*	Of little or no importance	Uncommon
Taeniorhynchus africanus	Of little or no importance	Uncommon

Source Lewis (1943)
* *Not a proved potential vector*

However in interpreting his conclusions it should be borne in mind that they were drawn from information secured the year following the epidemic and it is conceivable that the relative prevalence of the potential vectors may not have been the same while the epidemic was in progress Nevertheless the possibility of *A vittatus*, particularly and perhaps some of the other aedes besides *A aegypti* having participated as vectors in this epidemic cannot be denied As *A vittatus* and the other aedine species that Lewis believed may have been important vectors in the Nuba Mountain epidemic are distributed widely in East and Central Africa it is conceivable that they may be responsible for transmission in other localities in this region

BIONOMICS OF PROBABLE AFRICAN VECTORS

A brief statement on the breeding and feeding habits that affect the fitness of the two proved vectors of yellow fever in Africa (*A aegypti* and *A simpsoni*) and several of the suspect vectors will not be out of place here

The ability of insects to change their habits to meet the challenge of environment is a well recognized natural phenomenon This phenomenon is illustrated by *A aegypti* It has been suggested that this mosquito was originally a tree hole breeder and zoophilic in its feeding habits But from

association with man it changed in some regions to a highly domesticated type breeding almost entirely in artificial water containers about human dwellings and depending largely upon man for its blood meals. Be that as it may, gradations between these extremes are found today. In the Americas, as previously described, is found the highly domesticated strain with its ecology intimately tied to the human economy. In Uganda it is mainly a sylvan tree-hole breeder and essentially zoophilic. Though widely distributed through the forests, it is not numerous and is rarely found about towns or villages (Haddow, 1945a). It seems to play little if any part in the transmission of yellow fever virus either in the forests or among human populations.

In West Africa the habits of *A. aegypti* more closely simulate those of the American strain, but there the mosquito is not so dependent upon human cooperation in furnishing convenient breeding receptacles as it is in America. While it is most abundant about houses and is definitely homophilic in its feeding preferences, two characteristics that admirably qualify it as a vector, it is not so exacting in selecting places to deposit its eggs nor is it so restricted in its habitat. Its larvae are found not only in artificial water collections, such as those in clay pots and jugs, but also in tree holes and bamboo sections and even crab holes at distances up to 500 yards from the nearest house (Dunn, 1927*a*, *b*, *c*, and 1928). Though they are the most common potential vector found within houses, the imagoes have also been captured in neighboring brush and forests. These more cosmopolitan habits do not detract from aegypti's vector proficiency but do render its control much more difficult and virtually preclude the possibility of eradication. Nevertheless, the reduction of artificial breeding sites through the installation of piped water supplies seems to have had a measurable influence upon the incidence of yellow fever (Beeuwkes and Mahaffy, 1934).

In the Anglo-Egyptian Sudan, with its limited and seasonal rainfall and the necessity of storing water in earthenware receptacles and rock holes near houses, *A. aegypti* is again found as a partially domestic mosquito and a potential vector of yellow fever.

This varied behavior is related directly to, if not molded by, the environment. In Uganda, where there is frequent and heavy rainfall throughout the year and an abundance of surface water, the storage of water in receptacles for human use is unnecessary and there are few attractive breeding sites about houses. Also the temperature range in much of the upper mountain rain forest area of Central Africa may be rather low for the plentiful

propagation of this mosquito On the west coast not only are the climatic conditions favorable but the custom dictated by necessity of storing water about houses creates an inviting atmosphere for the domestic strain of *A aegypti* The even more domesticated American strain likely stems from West Africa

In Uganda *A simpsoni* the proved vector of yellow fever breeds chiefly in axils and was found in bananas *pineapples Dracaena and particularly* in Colocasia a widely cultivated food plant It was not found inside houses but in their immediate vicinity it was present in significant numbers and comprised 97 per cent of the mosquitoes captured (Mahaffy *Smithburn et al* 1942) *It feeds in the morning and late afternoon and* prefers human blood to that of goats fowls and monkeys (Haddow 1945*b*) It may venture for short distances into neighboring forests Because of these habits it is suited to transmitting the infection not only from man to man but also from marauding monkeys to man It occurs on the west coast but it is not abundant and is not regarded as an important vector in that region

In the Nuba Mountains *A vittatus* was observed breeding principally in rock holes though a few larvae were noted in jars It was found biting in the vicinity of but not within houses *A metallicus* is a tree hole breeder with presumably a short flight range No data on breeding sites were given for *A furcifer* and *A taylori* in the Nuba Mountain area but many adults were found biting out of doors in the evenings (Lewis 1943)

EPIDEMIOLOGIC TYPES OF THE MAN—MOSQUITO CYCLE IN AFRICA

As the elements for both the forest cycle and the man—mosquito cycle of yellow fever coexist over large parts of Africa where immunity surveys have demonstrated the occurrence of the disease and as the virus is interchangeable between these two cycles it is frequently impossible to decide which cycle is responsible for the maintenance of the virus and the endemicity of the disease in man At this point only the man—mosquito cycle will be discussed

Immunity surveys have indicated that the incidence of yellow fever is in general higher in West Africa particularly along the coast than elsewhere on the continent (Beeuwkes and Mahaffy 1934 Beeuwkes Mahaffy et al 1934 Sawyer and Whitman 1936 also see Table 30 page 580) It is believed these high immunity rates which in Nigeria for example average

25 per cent of the population surveyed, are attributable to the rather de population and particularly to the prevalence of an efficient domestic tor—*A. aegypti*. In this region studies have extended over a longer per than elsewhere and the information on the behavior of the disease, thou far from complete, is more extensive. It seems safe to assert that the dise is regionally endemic and locally epidemic. That is, over wide areas it constantly present, and within these areas periodic outbreaks occur tha attain epidemic proportions. There may also be epidemic thrusts beyond the area included in the endemic zone.

It can be assumed that the cardinal requirement for endemicity of the man–mosquito cycle in West Africa is the more or less continuous year round presence of *A. aegypti*, which in turn is dependent upon climate, temperature and rainfall, and suitable breeding places. Where these conditions are fulfilled, endemicity may be expected, and where they are not the virus is unable to persist in the man–mosquito cycle. The endemic zone for this cycle cannot be accurately defined, but it probably extends from Senegal southward along the coast to Angola. Ramsey (1931) concluded that the climatic conditions in Senegal were not propitious for endemic aegypti transmitted yellow fever, and placed this country in the epidemic rather than the endemic area. The extension inland of the endemic zone varies in different parts, but in general it is dependent upon the altitude, temperature gradients, rainfall and the habits of the people of the locality. Thus it is limited by the mountains when an altitude of from 1 000 to 5 000 feet is reached, and by the grassland and desert area to the northeast where conditions are not favorable to the continuous and adequate propagation of aegypti.

The designation of an epidemic within the endemic zone is a matter of degree, that is, the incidence of the disease at any given time. When the incidence is excessive, it is defined as an epidemic. In practice this may depend upon whether or not it reaches the attention of the authorities, as the disease may be quite prevalent in the native population without attracting notice. Indeed, in the past it was usually only when the European residents were affected that yellow fever was recognized. Notable epidemics have occurred periodically in large urban centers, as for example in Lagos 1925, 1926, Bathurst 1934, Accra 1926–1927, and again in 1937, and more recently in Ogbomosho 1946. Likewise, similar outbreaks have been observed in smaller communities (Findlay and Davey 1936*a* and *b*). The exact reason for these epidemic outbreaks is not precisely understood, but

they are probably due to a combination of factors—the proportion of tibles among the population, the temporary density of aegypti, and troduction of the virus. If the virus is introduced into a locality wh sufficient proportion of nonimmune persons exists and where there i quate density of aegypti, a chain of infections may be initiated that cause the disease to reach epidemic proportions. If the conditions of sp are not so favorable, the disease smolders or is limited to a restricted ne borhood and escapes notice.

Findlay (1941) distinguished three epidemiologic types of yellow fev in Africa: (*a*) urban epidemicity, (*b*) rural epidemicity, and (*c*) rural e demicity. He would place the responsibility of endemicity upon rural se tions and presumably exonerated the cities and urban communities. It i questionable, however, if this restriction is justifiable. Certainly it is not in accord with the deductions reached by Beeuwkes and Mahaffy (1931) from their immunity surveys. These surveys showed that in endemic cities the percentage of immunes increases gradually with age, which is indicative of endemicity. Findlay, in arriving at his conclusion, may have been influenced by the apparent absence of yellow fever during the intervals between epidemics; but as Beeuwkes (1936) has stated, yellow fever is rarely recognized in the native, and the relatively small number of cases reported in Europeans gives no reliable indication of the incidence or distribution of the disease. Analogy may be drawn with the former situation in northeastern Brazil, where, before antilarval control was instituted, yellow fever was regionally endemic and involved not only the rural sections (small villages) but the cities as well. Ridding the cities of *A. aegypti* did not eliminate the disease; it continued to be endemic in the rural area. But until the vector is effectively controlled in cities—a condition not yet attained in most African cities—it is not reasonable to disregard completely the participation of urban communities (cities) in the retention of the infection. This does not necessarily imply that the virus is constantly present in any one urban district, but, as in villages, it may come and go, and through a process of irculation, the virus being carried by infected individuals, a state of re ional endemicity is created.

There is a source other than the man–mosquito cycle that may con ibute to rural endemicity, namely, the introduction of the infection from forests. This is evidently what Findlay had in mind in designating rural lemicity. The occurrence of this event will be discussed after the forest e of the virus has been described. It may be remarked in passing, however

that in rather densely populated areas such as exist along the west coast of Africa and where the domesticated type of aegypti is widely disseminated it is not necessary to invoke any other explanation of the endemicity of yellow fever than the presence of this mosquito Before accepting the likelihood of other means of seeding the virus it would be required to rule out the possibility that aegypti may be involved In any event it is highly probable that this mosquito and the man–mosquito cycle is responsible for the majority of human infections over a great part of West Africa

In only two other regions in Africa has sufficient information been collected to warrant forming an opinion regarding the manner of transmission of the virus from man to man The Nuba Mountains epidemic of 1940 in the Anglo Egyptian Sudan described by Kirk (1941) is of unusual interest first because it is the largest epidemic ever described in Africa and second because it occurred in a so-called silent area Previous immunity surveys (Hewer 1931 Sawyer and Whitman 1936) had shown the rather wide distribution of immunity in this general area ranging from 0 up to 80 per cent in different villages The epidemic of 1940 broke rather suddenly and was not anticipated because yellow fever had not been recognized clinically in this area The only suspected contributing factor was the unusual movement of population and the introduction of a number of non immunes into the area preceding the outbreak Neither the source of the infection nor the factors that promoted its epidemic spread can be defined with certainty The epidemic seemingly commenced in an isolated mountain village and was thence spread to other villages by infected individuals Before the epidemic subsided more than 15 000 cases and 1 500 deaths were reported However in comparing previous and subsequent immunity rates in a number of villages it is estimated that the number of infections may have reached 10 000

The question of vectors involved in this epidemic has been alluded to lready and will not be further discussed here but it is quite evident that the epidemic represented a man–mosquito cycle of the disease According o Kirk it is difficult to avoid the conclusion that the epidemic actually riginated in the Nuba Mountains and was not introduced from West frica or from elsewhere If this conclusion is correct it must be assumed at yellow fever is endemic in the Anglo Egyptian Sudan

Two possibilities of the maintenance of the virus in this region must be nsidered First maintenance in the man–mosquito cycle and second intenance in some animal mosquito cycle As *A aegypti* as well as

other vectors found about houses are numerous in this region the conditions for the man–mosquito cycle endemicity seem to be rather favorable It is true that during the dry season most of the mosquito breeding ceases but according to Kirk *A aegypti* is an exception to this rule and is found in considerable numbers all the year round in some villages where domestic supplies of water are preserved in unprotected containers Though a number of species of primates are found to inhabit this region no adequate investigation of the existence of the forest cycle of the virus has been made in view of the continuous presence of *A aegypti* throughout the year and the presence of other potential vectors about houses during the rainy season the most likely explanation for the maintenance of the virus is by means of the man–mosquito cycle However before accepting this hypothesis the possibility of the existence or an extension of the forest cycle into the region should be investigated

The first instance definitely incriminating a mosquito other than *A aegypti* in the transmission of yellow fever from man *to* man in Africa was reported by Mahaffy Smithburn et al (1942) from Bwamba County Uganda Periodic immunity surveys of the village inhabitants had demonstrated that infection with yellow fever was current The authors during an intensive study succeeded in recovering yellow fever virus from a batch of *A simpsoni* captured in the vicinity of the houses and from a woman living in the village Since simpsoni constituted 97 *per cent* of all mosquitoes captured in and around houses it seemed evident that simpsoni was the vector responsible for transmitting the infection *A aegypti* is rare in this region and only one specimen was captured during the course of the study While the mosquito–man cycle with *A simpsoni* as the vector was apparently demonstrated in this locality this cycle was evidently not responsible for the continued maintenance of the virus because 11 months after effective mass immunization of the human population the virus was again isolated from *A simpsoni* At this same time virus was also isolated from a mixed lot of mosquitoes of the genus *Aedes* captured in the uninhabited Semliki forest of Bwamba County (Smithburn and Haddow 1946) The role of *A simpsoni* as an intermediate vector between the forest and the human cycle of the virus will be discussed later

Immunity surveys have shown that yellow fever exists in East and South east Africa as far south as northern Bechuanaland (Mahaffy Smithburn and Hughes 1946 Smithburn Goodner et al 1949) (see Table 30 p 580) The epidemiology of the disease however is as yet unknown since no ade

quate field studies have been conducted either as concerns the potential vectors or the animal hosts other than man

THE FOREST OR ANIMAL MOSQUITO CYCLE

A phase in the natural history of yellow fever virus that is more complicated and probably more fundamental from the aspect of the virus than its passage through man is its life in the forest. As with many discoveries adduced from the accumulation of suggestive clues, it is difficult to determine when the concept of the forest cycle arose and when the proof was sufficiently convincing to make it generally acceptable.

The first specific suggestion that monkeys might constitute a natural reservoir of the virus seems to have been made by Balfour (1914) following a trip to Trinidad. He recounted a legend common among the natives that howler monkeys were observed to be sick or dead in the forest preceding epidemics of yellow fever, and he cited the history of a small epidemic among employees in a camp of a petroleum company, in which the first two men to become ill had been working at an oil bore situated at the end of an 8 mile road recently cut through a dense virgin forest. In retrospect it is probable that the observations of the natives were correct and that the two persons were infected by contact with forest mosquitoes. However, such scanty evidence, though suggestive, is not convincing, particularly as *A. aegypti* were present in the camp and no doubt accounted for the subsequent cases.

Preceding this hypothesis of Balfour's, Franco, Martinez Santamaria and Toro Villa (1911) had investigated an epidemic in the region of Muzo, Colombia, which according to carefully noted symptoms appeared to be yellow fever. The following conclusions were drawn regarding the disease:

(*a*) It is contracted in the forest and not in the neighborhood of the houses. (*b*) It is transmitted by *Stegomyia calopus* (*A. aegypti*) and probably also by other culicines. (*c*) Inoculation takes place during the daylight hours which are spent by the workers in the places where the transmitting mosquitoes predominate. (*Translated from Spanish.*)

The implied presence of *Stegomyia calopus* (*A. aegypti*) was probably an error in identification, as subsequent searches in the region failed to reveal existence of this mosquito.

Later in 1916, when suspected cases were again reported from Muzo

the region was visited by the Gorgas Commission (Gorgas 1917) and as they were unable to find any *A aegypti* mosquitoes after careful search it was decided that yellow fever had not been present and that the cases reported had probably been malaria The conclusion of the commission is of interest as it reveals the attitude then prevailing—that the sole transmitter of yellow fever was *A aegypti* and in the absence of this mosquito yellow fever could not exist

Following the transmission of the virus to monkeys in West Africa the demonstration that it could be conveyed by mosquitoes other than *A aegypti* and the proof of the susceptibility of African and South American primates the possibility that the virus might exist in some cycle in which man is not involved must have arisen in the minds of many workers then engaged in yellow fever research This concept was expressed by Davis (1930*b*) who said

> The fact that the virus of yellow fever can be passed through marmosets with comparative ease may have some importance in the epidemiology of the disease in nature These little monkeys are frequently kept as houshold pets and even the wild ones are often found on uncleared land within urban limits It is conceivable that they might pick up yellow fever from domestic sources carry it to outlying districts and aid in its wide dissemination Such a course of events seems the more possible since we know that various wild species of mosquitoes are fairly efficient vectors of the virus

In 1933 Soper Penna et al presented indubitable evidence of the existence of yellow fever in the Valle do Chanaan in the State of Espirito Santo Brazil in the absence of *A aegypti* There was no doubt of the diagnosis as it was confirmed by the isolation of the virus by histopathologic examination of a liver specimen and by neutralization tests on paired sera Also the absence of *A aegypti* in the area covered by the epidemic cannot be questioned as a thorough search for this mosquito was made by experienced observers The importance of this observation upon the control of yellow fever cannot be overemphasized but at the time its true significance was not fully appreciated as the authors stated This epidemic was apparently self limited and it is possible that there are no rural regions in America which are truly endemic for yellow fever There was also no reference made to a suspected forest cycle of the virus

At a conference in November 1934 Soper (1935*a*) alluded to the possible existence of vertebrate hosts other than man but it was not until the following year that he in a lecture given before the faculty of medicine at

Bogotá (Soper 1935b) first used the term jungle yellow fever to designat[e] infection in man acquired as a result of forest contact in the absence o[f] A. aegypti. He referred in this instance to observations of Boshell Manrique on the occurrence of yellow fever in the district of Restrepo, Colombia, among natives working in fields near forests where vicious biting blue mosquitoes were particularly prevalent. There were no aegypti in the region and four of six blood specimens taken from monkeys gave positive neutralization tests. In 1936 additional evidence (Soper 1936a and b) was published on protection tests in monkeys which indicated that monkeys deriving from regions where yellow fever was known to exist frequently showed specific immunity to the virus while sera of those derived from places where yellow fever was apparently absent gave negative neutralization tests. By this time the concept of a forest cycle of the virus had been pretty generally accepted, as it had been shown that yellow fever is contracted in isolated forested regions through contact with the forest and in the absence of A. aegypti, that a number of forest mosquitoes, including Aedes leucocelaenus, Aedes scapularis and species of haemagogus, are capable of transmitting the virus from monkey to monkey, and that primates of several species captured in the forest were specifically immune to yellow fever. Additional and perhaps clinching evidence of the propagation of the virus in the forest was contributed by Shannon, Whitman and Franca (1938) when they were able to infect monkeys by the bite of forest mosquitoes of the species Haemagogus capricornii and A. leucocelaenus that had been captured in the jungle. Finally, a number of years later, Laemmert and de Castro Ferreira (1945) succeeded in obtaining yellow fever virus from captured wild-caught marmosets.

In Africa in the meantime primates in their natural state were shown to possess immunity to yellow fever virus and subsequently the virus was isolated from mosquitoes deep in uninhabited forests and from sentinel monkeys stationed in the forests. (For references see under Forest Cycle in Africa.) This array of evidence led to the inescapable conclusion that in both South America and Africa the virus of yellow fever is maintained in a forest cycle independent of man.

METHODS USED IN FOREST STUDIES

When it became suspected that there was an extrahuman cycle of the virus and the trail led beyond the environs of human habitations and into

the forests the problem surpassed the competence of medical epidem
gists and virologists It became necessary to obtain the aid not only of e
mologists but also of naturalists trained in the sciences of mammalogy
nithology and ecology for it was conceivable that any of the great var
of vertebrates and arthropods found in tropical forests might serve as ho
or vectors of the virus This endeavor brought into play the methods a
techniques that the several sciences had to offer In the study of no oth
disease has so much work been done on the sylvan fauna of tropical Sout
America and Africa In the course of these studies thousands of vertebrate
and hundreds of thousands of arthropods were captured classified and ex
amined The object was to trace the virus through its hosts and vectors in
order to determine by what means it was being maintained in the forests
and how it was being transmitted to man

In domestic animals the existence of disease usually can be recognized by illness (clinical manifestations) or death These visual means are not applicable in wild forestral animals Occasionally dead howler monkeys have been reported in the forests of South America preceding yellow fever outbreaks in nearby human populations but these instances are rare and of no practical value in identifying the presence of yellow fever virus in forests Few African monkeys and only some of the species of South Ameri can primates succumb when infected and none remain ill beyond a few days Also the carcasses particularly of the smaller species may be rapidly devoured by carrion birds or mammals Even with constant vigil which has been exercised in the study of local epizootics the chance of recognizing the disease by observing sick or dead animals is remote It was therefore neces sary to resort to other means

From the laboratory as mentioned before had evolved two invaluable
aids to this objective without which efforts would have been futile These
are (a) means of detecting the virus—by inoculation of monkeys and mice
and (b) means of detecting previous virus infection of an animal—by the
neutralization and complement fixation tests While both monkeys and
mice were used for isolation of the virus the former are preferable be
cause larger quantities of suspect material can be inoculated and they are
susceptible to infection by the bite of an insect Thus by using monkeys
it is possible to determine whether the virus is present in wild caught mos
quitoes and also whether or not the insects are actually transmitting the
infection by bite For detecting immunity the complement fixation test

was used to some extent, but the virus-neutralization test proved more reliable and was much more extensively employed.

Both the direct and indirect approach were resorted to with the hope that the evidence accumulated by the one would complement and support the evidence accumulated by the other. The direct approach refers to efforts made to isolate the virus and to trace its passage through animals by the immunity it is known to confer. These efforts comprised (*a*) search for the virus in arthropods by inoculating triturated suspensions of them into susceptible animals or by allowing the arthropods to feed upon suitable susceptible animals; (*b*) search for the virus in captured animals by inoculating their sera or suspensions of their organs into other animals known to be susceptible; (*c*) stationing sentinel monkeys in forests to see if they became infected; (*d*) testing for specific neutralizing antibodies in the sera collected from forest animals; and (*e*) capturing, labeling, releasing, and recapturing monkeys and testing them for immunity on each recapture. If there were any human inhabitants in the vicinity of the forests, they were tested for immunity, sometimes periodically, and attempts were made to isolate the virus from those developing suspicious symptoms.

The indirect approach refers to the study of the environment—the flora and fauna—conducive to the maintenance of the virus. This involved (*a*) a survey of the flora of the region; (*b*) a survey of the arthropod and animal population and the relative prevalence of the different species of each; and (*c*) a study of the ecology of species of arthropods and animals, particularly of those suspected of being involved in the cycle of the virus. Again, if there were nearby human inhabitants, their collective or individual habits in relation to their immunity to yellow fever (previous infection) were investigated.

It was soon suspected that the virus did not remain indefinitely at any fixed place, and one of the problems in choosing a site of operation was to ascertain whether the virus was actually present within the area. In South America the establishment of viscerotomy posts was most useful in this respect. Otherwise, and this applied particularly to Africa, it was necessary to rely upon clinical manifestations of the disease among inhabitants living near the forest or upon periodic immunity surveys for revealing whether or not infection was taking place during the interval between the surveys.

As is often the case, it was necessary to modify existing methods or

develop new ones adapted to this type of investigation a brief description of those found most useful will be given For detailed information see Bugher Boshell Manrique et al (1944) Taylor and Fonseca da Cunha (1946) Laemmert de Castro Ferreira and Taylor (1946) Haddow Smithburn et al (1947) Haddow and Mahaffy (1949) Smithburn Haddow and Lumsden (1949) Depending upon circumstances and the accessibility of the forest to be investigated one of two procedures was followed either a field laboratory was set up in the vicinity where the primary inoculations were made in searching for virus the animals were bled and the serum was conditioned for transportation to the base laboratory or if it was not feasible to establish a field station in or near the forest a mobile unit for collections and even initial inoculations was sent into the suspected area

It was not feasible to apply both the direct and indirect approach in all instances The completeness of the surveys at any given time and place was dictated by the local circumstances and opportunities When a clue was received indicating that the virus was active in a locality it was frequently expedient to organize a field excursion quickly and to focus attention upon the most promising lead In other instances a watch and wait policy was adopted A field station was established in an area where the virus was thought to be endemic and the observations were pursued over a longer period with the hope that the circulation of the virus would become sufficiently active to be detected somewhere within the area This type of study permitted collection of more comprehensive data As a rule however, all of the investigations included a survey of both vertebrates and arthropods

Out of these forest studies and from the laboratory have emerged facts which make it possible to piece together a plausible story of at least one forest cycle of the virus These facts together with suggestive information are now presented Later the question whether or not this knowledge offers an acceptable explanation of the natural history of the virus within forests will be discussed

Collection of Vertebrates The manner of collecting vertebrates varied according to the species sought and the prevailing local conditions In South America the trapping of most animals proved to be feasible For this purpose traps of various types and sizes were set in the forests along trails Traps that did not kill or injure the animal were obligatory only occasionally were kill traps employed and then only for sampling purposes to ascertain the species of the animal present in the area A description of traps successfully used is given by Gilmore (1943) These varied in size from the small tin can trap up to the larger stockade

type constructed on the site for capturing monkeys The trap line was run in the morning and the afternoon, the captured animals were removed and the trap was reset with new bait

Where trapping was unsuccessful especially in Africa it was necessary to resort to other means of obtaining blood specimens such as shooting animals or felling trees in the hollows of which they were nesting The latter method was



Fig 91 A mosquito-catching station in Colombia

particularly useful in securing specimens of small nocturnal primates The blood ... were subjected to the neutralization test and in some of the studies the ...sh sera were subinoculated into susceptible primates

...ollection of Arthropods Among the free living arthropods greatest attention ... focused upon mosquitoes as it had been proved by laboratory experiments ... several species of forest mosquitoes were effective vectors of the virus How... many other arthropods were collected and examined for virus ...squitoes were collected on the ground and at varying elevations in trees ...was observed that the relative prevalence of different species of mosquitoes ... markedly with the elevation Platforms reached by ladders were con...d in some trees while other trees were scaled with the aid of climbing irons ... it was desired to make comparative studies extending through several sea

sons trees were selected and captures made in the same trees and under the same conditions periodically Most of the captures were made freehand the collector serving as bait though some mass collections were made by using traps described by Shannon (1939) and a mechanical trap described by Mulhern (1942) Mosquitoes containing blood were discarded in order to avoid the possibility that the virus might be neutralized since all of the collectors had been vaccinated against yellow fever However an experienced capturer would usually take the mosquito before it had opportunity to withdraw blood In the beginning Simplex suction tubes were employed for hand captures but in South America this method was later abandoned in favor of small flat bottom tubes capped with a snugly fitting cup of Monel wire mesh The bottom of each tube contained a piece of moist blotter paper The mosquitoes were captured individually in these tubes which were then placed in wooden racks for transportation to the laboratory This method avoided transfer of the mosquitoes and facilitated their classification Also the mortality during transportation was reduced to a minimum The mosquitoes usually arrived at the laboratory within 24 hours after capture for inoculation or for feeding upon susceptible monkeys Hourly records of meteorologic conditions were kept and the hour of the capture of each mosquito was noted on the container The foregoing description applies particularly to the methods latterly used in South America (Bugher Boshell Manrique et al 1944 Bates 1944*b* and Laemmert de Castro Ferreira and Taylor 1946) In Africa they were essentially the same varying only in minor details (Haddow Gillett and Highton 1947 Haddow and Mahaffy 1949)

Ectoparasites were obtained by wrapping the animal in a white cloth placing it in a jar containing ether and when the animal was under anesthesia removing it and combing its fur Parasites present about the ears and eyes of the animal were removed with forceps The parasites were then collected from the white cloth and placed in test tubes for later classification and examination for virus The mosquitoes were examined for virus either by permitting them to feed upon a susceptible monkey or by inoculating triturated suspensions of their bodies Not infrequently both procedures were applied in sequence upon the same batch of mosquitoes Arthropods that do not readily feed upon monkeys such as ectoparasites from other animals were ground in a suitable diluent and the suspension was inoculated subcutaneously

Collections and Observations of Birds The collection of birds as a rule proved a more difficult task than the collection of mammals Forest birds are exceedingly wary and resist capture Most of the birds were obtained by shooting By using shot of proper size and taking care not to mutilate the specimen by shooting at too close range satisfactory blood specimens could be secured from over 80 per cent of the birds of all sizes that were shot As with shot mammals the blood specimen was obtained for the most part by opening the chest wall and plunging

a needle directly into the heart For more detailed description see Laemmert de Castro Ferreira and Taylor (1946)

Sentinel Monkeys It was thought that a good way of detecting presence of the virus might be to place normal susceptible monkeys in a large mesh wire cage and station them within forests This procedure was used to some extent in South America and more widely in Africa without success The fact that the monkeys did not become infected was not understood for in many of the forests where they had been placed there was a high rate of immunity to yellow fever among the wild monkeys and there was reason to believe that the virus was actually circulating within the forests After a few trials the method was abandoned in South America but in East Africa investigators were more persistent and ultimately solved the mystery of why the sentinel monkeys had not become infected It was discovered that the species of mosquito transmitting the virus in East Africa would not enter the cages although these were constructed of wide mesh expanded metal When this became known and monkeys were placed on platforms without being protected by a cage a number of them developed infections and virus was isolated (Smithburn Haddow and Lumsden 1949) This instance and there are many others that might be cited is illustrative of how a simple change in technique may alter the result It may be remarked that the reason for initially placing the monkeys in cages was to protect them from predatory animals particularly eagles Some uncaged monkeys were lost but the number was not sufficient to render the procedure impractical Whether or not the apparent failure to obtain infection of sentinel monkeys in South America was due to their being placed in cages is not known as it was not definitely determined whether the forest vectors in South America were as wary of entering cages as those in East Africa

The temperature of the sentinel monkeys was ascertained twice daily and if there was a significant rise a blood specimen was taken and inoculated intracerebrally into white mice and or a nonimmune rhesus monkey The sentinel monkeys were also tested at intervals to see whether they had developed immunity

A modification of the sentinel monkey method was latterly used in Brazil Cebus monkeys were captured in selected forests and tested for immunity and the nonimmunes were labeled and released When recaptured they were again tested for immunity and again released It was found possible to recapture the same animal time after time Although no virus activity was detected in the area under study it was demonstrated that this method may be useful in forests where Cebus monkeys abound (Laemmert Hughes and Causey 1949)

Ecologic Observations A number of studies were made on birds and also on animals with ecology primarily in mind (Davis 1945a and b 1947) and rather extensive observations were made in both South America and Africa on a number

of species of mosquitoes which had been shown capable of transmitting the virus experimentally. In all the field studies, however, some attention was given to the prevalence and ecology of vertebrates and hematophagous arthropods found in the forests, especially of those which for one reason or another were suspected of playing a part in the forest cycle.

Analysis and Interpretation of Data. Two approaches, or a combination thereof, may be used in sifting evidence in an attempt to understand the natural history of an infectious agent: (*a*) weighing positive evidence with the purpose of deciding whether it offers a satisfactory explanation for the observed mass behavior of the infection, and (*b*) eliminating on the basis *of negative evidence other factors (hosts and vectors) that might be* concerned.

Since negative evidence is rarely exclusive, and since in this instance it would be virtually impossible to examine exhaustively all the conceivable forestal hosts and vectors, greater stress will be laid upon the positive evidence. It is believed, however, that due weight should be given to some of the negative evidence that has been accumulated, particularly by laboratory experimentation. It is difficult to conceive, for example, that an animal found to be resistant to experimental infection with the virus or an arthropod that will not retain or transmit the virus under the most favorable *laboratory conditions is likely to play a part in its natural propagation.* Hence it is proposed to eliminate from consideration vertebrates and arthropods that fall into this category.

A definition of epidemiologic susceptibility of hosts and vectors has been given, but it may be well to restate in another manner the criteria that determine their capacity and efficiency as vectors and hosts in nature. To paraphrase Soper (1945): For an insect to be important in maintaining and disseminating the virus it should be (*a*) susceptible to infection and able to transmit the virus by bite, (*b*) sufficiently long-lived under natural conditions to survive well beyond the extrinsic incubation period of the virus, (*c*) in nature exposed to intimate contact with the source of the virus and with nonimmune susceptible vertebrates, and (*d*) present in adequate numbers. An animal, in order to receive consideration as an important host, must be (*a*) numerous in the infected region, (*b*) susceptible to infection by the vector and capable of circulating large amounts of virus (sufficient to infect the vector) in the peripheral blood stream, and (*c*) acceptable and accessible to local vectors as source of blood meals.

e forest virus may invade any type of forest that harbors the sts and vectors its permanent abode is seemingly associated with e rain forest At least the zones in which the infection is known ally endemic are characterized by this type of forest It is there er to describe briefly some of the distinguishing characteristics this nature

iting such a description one is discouraged by the complexity of ment As Bates has remarked You can never hope to know the est It is too complex too endlessly varied in its details built of ifferent kinds of organisms for any man to gather more than a acquaintance Nevertheless there are certain characteristics pe opical rain forests that help to distinguish them from forests in ite zone and other types of forests within the tropical belt In complexity and the almost infinite variety of flora and fauna ipport is in itself a distinguishing feature It would be a monu to describe even casually the known living inhabitants of these shall therefore confine ourselves to a few environmental features ect the mechanism of the cycle of yellow fever virus within them nd description of forest environment Allee Park et al (1949) oted

our attention from the over all interplay between climate and com the application of principles of stratification to forest community on we find that these communities typically have six basic vertical ns (1) subterranean (2) floor (3) herbaceous (4) shrub (5) tree and of air above the canopy All six are present in most forests All are ndent each has a particular microclimate and a particular biota ject to and reponds characteristically to extraforest and intraforest nd biological forces

the first five strata may be substratified This is increasingly true xceeds from pioneer to climax communities For example the forest Colorado Island in the Panama Canal Zone has been shown to have ta (1) the air above the forest (2) tree tops above the main forest or more feet high (3) upper forest canopy 75 to 100 feet high (4) e tops (second story trees or midforest) 40 to 60 feet high (5) small to 30 feet high (6) higher shrubs 10 feet high (7) forest floor and rranean according to Allee (1926a) The herbaceous stratum in

such forests is poorly developed On the other hand the herbaceous layer is often well developed in north temperate deciduous forests

Epiphytes especially in tropical forests may swell the mass of available habitat space Tree trunks with their ancillary branches and vines form more or less vertical highways between the several strata of the forest and are extensively so used by forest animals Similarly the floor with its vertical discontinuities such as tree holes and second floors adds to the complexity

Certain general principles may be noted The vegetation forms the primary biotic gradient in terrestrial communities Stratification serves to increase the organic volume of the community and hence increases kind and amount of shelter and foods directly for herbivores and indirectly for carnivores and carrion feeders and dung feeders It follows that the taxonomic complexity of the community increases with its increase in stratification The process of stratification is intensified as the ontogenetic age of the forest increases Stratification usually increases in direct proportion to the seral age of the forest Finally it follows that stratification becomes a criterion of both actual and relative maturity

One of the characteristics of tropical rain forests is the more or less continuous top coverage of foliage known as the canopy There is a constant struggle for light and only the taller trees achieve the top of the forest The less fortunate ones or those requiring less light spread their leaves at varying distances underneath The base of the forest with the exception of a scattering of young trees or palm shoots struggling for survival and some ferns lianas and parasitic growth on fallen trees may be largely devoid of leafy vegetation One can usually walk within these forests without great inconvenience until the edge is reached Here as a result of the infiltrating light is met a dense impenetrable barrier of brush and vines It is as if the top of the forest had dipped downward to meet the ground

The canopy is the first conditioning factor of the environment of the underlying strata It shuts out all or nearly all of the direct rays of the sun and only subdued light filters through to the ground creating the impression of a still damp twilight Though a storm may be raging above there is little air movement near the floor of the forest The temperature and humidity remain exceedingly constant Passing upward through the lianas that cling to the trunks of the trees and the epiphytes that spring from the branches a stratification of microclimates is produced This environmental stratification results in a variation of the fauna The animal and insect life attempts to choose the environment to which it is best adapted some species

preferring to dwell near the ground and others at different levels in the trees, but perhaps the most favored place is in the forest canopy. Bates (1945a) has drawn a comparison with the sea and the changes in marine life which vary according to the depth below the surface.

These rain forests are very ancient, which may account for the great diversity of plant and animal life—another distinguishing feature. For example, Bates stated that a greater number of mosquito species was found in an acre of Colombian rain forest than exists in the entire United States. There is only one species of marsupial north of the Mexican border, while a dozen or more, varying in size from a mouse to a cat, live in the rain forests of South America. There are many other examples of the exuberance of animal life. The same applies to the vegetable kingdom. In the temperate zone and in second growth forests, the species are limited and there are many examples of the same species, while in the climactic tropical forests there is an enormous variety of species with relatively few examples of each.

Rain forests in different continents and in different localities in the same zone differ greatly in detail, but in general the environmental characteristics described above apply to all. The features that have a direct bearing upon the natural history of yellow fever virus are the vertical stratifications of the environment and their effect upon the ecology of the hosts and vectors, as it is obvious that the relation is most intimate among the arthropods and vertebrates inhabiting the same stratum.

PROBABLE FOREST VECTORS IN SOUTH AMERICA

A list of references to the New World mosquitoes examined and shown capable of transmitting yellow fever virus is given in Table 7 (p. 263). There will be enumerated here only those shown able to transmit yellow fever virus and whose habitat makes it necessary to consider them as possible vectors in tropical and subtropical forests. These are *Aedes fluviatilis*, *A. scapularis*, and *A. leucocelaenus*, *Haemagogus spegazzinii* and subspecies *falco*, *H. capricornii*, *Haemagogus splendens*, and *Haemagogus equinus*, and *Trichoprosopon frontosus*. In addition, there is one other species, *Psorophora ferox*, concerning which evidence is equivocal. There are a number of additional species of the genus *Haemagogus*, found principally in Colombia and Central America, whose vector potentialities have not been studied. Since the species of this genus that have been tested were all

found capable of transmitting the virus the possibility of the unexamined species possessing this ability is not unlikely

There are other species of mosquitoes as well as a few species of other families of arthropods that may retain the virus for a time but are unable to convey it by bite These have not been mentioned as it is believed the mere temporary retention of the virus is of no epidemiologic significance

The frequency of isolation of the virus from wild caught mosquitoes combined with the prevalence and ecology of the species are of greatest epidemiologic import The virus has been obtained three times from *A leucocelaenus* and on 18 occasions from species of the genus *Haemagogus* (*capricornii spegazzinii* and subspecies *falco*) either by bite or by inoculation of suspensions of the mosquitoes While the number of isolations is small it may reflect to some extent their relative importance as natural vectors The unique isolation of virus from *Sabethini* was accomplished by inoculating intracerebrally into mice a suspension of a mixed group of 88 specimens Twenty one of the same group however failed to infect a monkey by bite In that only one species of the *Sabethini* *T frontosus* has been shown able to transmit the virus experimentally and it rather indifferently the implication of this single isolation is doubtful It may represent an instance of retention of the virus in mosquitoes that are unable to transmit it in nature Thus the only conclusive evidence of natural vectors supported by laboratory transmission applies to *A leucocelaenus* and to species of the genus *Haemagogus*

The ecology of the genus *Haemagogus* particularly the species *capricornii spegazzinii* and *spegazzinii falco* has been rather thoroughly investigated and is of considerable interest This genus of mosquito is widely distributed in the forests of South and Central America *H capricornii* occurs more frequently in the south and central part of Brazil *H spegazzinii* overlaps with *H capricornii* in central Brazil and extends northward along the coastal forests In the interior it is found in the great forests of the Parana and Amazon basins but as the Amazon River is approached other species of this genus appear *Haemagogus tropicalis* and particularly the *falco* subspecies of *H spegazzinii* the latter extending up through Colombia This distribution is deduced from random sampling but it seems evident that the habitat of mosquitoes of the genus *Haemagogus* covers the entire region over which the forest cycle of yellow fever virus has been recognized (Kumm 1950) These mosquitoes are essentially tree hole breeders but occasionally they have been found breeding near houses in artificial collec

tions of water. The adults are found in greatest abundance in the forest canopy. This characteristic, first noted by Boshell Manrique (Bugher, Boshell Manrique, et al., 1944), makes it necessary for anyone seeking to capture large numbers of these mosquitoes to climb close to the top of the forest. However, the mosquitoes may descend to ground levels, particularly along the forest edges. They appear to favor old or virgin forests in preference to the newer second growth type of forest (Laemmert, de Castro Ferreira, and Taylor, 1946). They are daylight feeders and more active at midday than in the late afternoon or early morning.

The habitat as well as the habits of *A. leucocelaenus* are somewhat similar to those of haemagogus mosquitoes. *A. leucocelaenus* is a forest mosquito, a day feeder and breeds predominantly in tree holes, but unlike haemagogus mosquitoes it shows no preference for the forest canopy. As a rule it is not so numerous as haemagogus, particularly in the upper strata of the forest, and in some forests harboring yellow fever virus it is exceedingly scarce (Laemmert, de Castro Ferreira, and Taylor, 1946; Kumm, 1950).

Though yellow fever virus has never been obtained from wild-caught *A. scapularis*, this species is an efficient experimental vector and was suspected of being involved in the epidemic of jungle yellow fever described by Soper, Penna, et al. (1933). It is not strictly speaking a forest type, as it is a surface water breeder, but it is found along the borders of and sometimes within forests. It is a day feeder and is captured largely on the ground instead of high in trees. Considering the habits of this mosquito, it is likely of little importance in transmitting the virus within the forests, although it may conceivably become infected from monkeys and transmit the disease to man.

PROBABLE FOREST HOSTS IN SOUTH AMERICA

In Chapter 6 information, particularly of an experimental nature, and notations on the habitat and ecology of the relatively susceptible animals have been given. It is now the intention to assess these data with the object of deriving an opinion on the vertebrates participating in the cycle of yellow fever virus in South American forests. By eliminating all of the vertebrates that have been found to be experimentally nonsusceptible, there are left for consideration the *Primates*, *Marsupialia*, a few species of the *Rodentia* and *Edentata*, and one species of *Carnivora*. These will be taken up in order of their presumed importance as hosts.

The evidence against the primates is beyond cavil Experimentally they are definitely susceptible they can be infected by the bite of mosquitoes they circulate virus in high titer mosquitoes may be infected from them and the virus can be maintained by alternate passage through them and a suitable mosquito vector Immunity from naturally acquired infection has been repeatedly demonstrated and the virus has been isolated from wild caught specimens (marmosets) Ecologically they fit into the picture of the known forest cycle of the virus They are widely distributed over the zone where forest contact infection of man is known to occur and there is a correlation of immunity in primates in the forests and in persons having contact with the forests (Soper 1936*a* and *b* Taylor and Fonseca da Cunha 1946 Laemmert de Castro Ferreira and Taylor 1946 Kumm and Laemmert 1950) Their habitat within the forest tends to bring them in contact with the proved vectors They are arboreal animals and spend most of their time in the upper forest levels near or within the canopy This is also the stratum in which there is the greatest abundance of the most important vectors—mosquitoes of the genus *Haemagogus* Ordinarily monkeys are very alert and should not be easy prey for mosquitoes but it is alleged that monkeys have the custom of napping during the midday hours which is the period of most active feeding of the vectors It is questionable however if the habits of the nocturnal species (*Aotinae*) favor their being fed upon by haemagogus mosquitoes as during the day these small animals are said to retire into tree holes From what is known of the behavior of haemagogus mosquitoes it is doubtful if they enter tree holes for feeding purposes As previously stated they are photophilic and seek the bright light but if on occasion these nocturnal monkeys sleep in exposed places as in a dense tangle of vines in the treetops (Enders 1935) or in the crotch of a limb they would be advantageously exposed to the vectors

Naturally acquired immunity has been observed in virtually all of the species of South American monkeys that have been tested in significant numbers This indicates that any of them may serve as natural hosts the importance of the role played by any particular species being dependent upon its relative abundance The greatest amount of information has been derived from special studies in Brazil and Colombia While the virus does not observe national boundaries it is probable that there is considerable variation in the epidemiologic pattern from one geographic region to another and though the mechanism may be similar the components in the cycle may vary widely

Bugher has assembled data on immunity tests done on captured animals in Colombia and although the number of animals examined is not so great as in Brazil they are of interest because most of the ones examined were captured or shot shortly following epizootics (Table 19)

TABLE 19

RESULTS OF IMMUNITY TESTS ON CAPTURED PRIMATES IN RESTREPO–VILLAVICENCIO REGION COLOMBIA, IN POSTEPIDEMIC AND EPIDEMIC PERIODS

Postepidemic period 1936–1937

Primates examined	*No pos*	*No neg*	*Total*	*Per cent pos*
Alouatta	12		19	63
Cebus	38	23	61	62
Ateles	14	1	15	93
Lagothrix	43	18	61	71
Aotus	1	0	1	
Callicebus	8	3	11	3
Saimiri		8	15	47
Total postepidemic period	123	60	183	Aver 67
Epidemic period 1940				
Cebus	9	1	10	90
Callicebus	1	0	1	
Ateles	8	0	8	100
Lagothrix	12	3	15	80
Saimiri	1	9	10	10
Total epidemic period	31	13	44	Aver 70

Source Bugher

Kumm and Laemmert (1950) have tabulated all the tests performed on primates in Brazil during the entire study conducted in that country As many of the blood specimens were collected in the process of sampling surveys with the object of determining the region over which the virus had spread and had no relation to known epizootics the immunity rates are considerably lower However in instances where specimens were collected shortly after an epizootic the rates were comparable to those obtained in Colombia (Table 20)

TABLE 70

RESULTS OF IMMUNITY TESTS ON PRIMATES IN BRAZIL

Genus of monkeys	*Number of sera tested*	*Number positive*	*Per cent positive*
Cebus (capuchin)	3,453	557	16.1
Aotus (night)	53	1	1.9
Pithecia (saki)	6	2	33.3
Saimiri (squirrel)	69	3	4.4
Alouatta (howler)	192	4	24.5
Callicebus (widow)	14	4	28.6
Ateles (spider)	10	6	60.0
Brachyteles (woolly)		1	14.3
Callithrix (true marmosets)	2,01[illegible]	103	5.1
Leontocebus (lion marmosets)	14	1	7.1
Marikina (tamarin)	27	0	0

Source Kumm and Laemmert 1950

It may be added that the immunity rates do not necessarily reflect the relative frequency of infection among different species as the infection may be fatal in some species and not in others Obviously the rates apply only to the surviving animals

The situation concerning the marsupials is rather confusing and contradictory The experimental evidence in reference to the susceptibility of this order of mammals has been reviewed in Chapter 6 The rather discordant results may be explained by the use of different strains of virus and perhaps gradations in susceptibility of the same species of animal in different regions Be that as it may only the genus *Metachirus* and some species of the genus *Marmosa* have been shown to be epidemiologically susceptible under laboratory conditions They may be infected by the bite of mosquitoes and circulate virus though generally in a lower titer than the primates also it is possible to maintain the virus by cyclic passage through these animals and mosquitoes (Bates and Roca Garcia 1946 *b* Waddell and Taylor 1948) However in Brazil immunity has been observed rarely in captured metachirus This discrepancy was particularly noticeable in a field study near Ilheus (Laemmert de Castro Ferreira and Taylor 1946) The strain of yellow fever virus isolated from marmosets during the course of this study was readily passed to metachirus and maintained without difficulty in this animal in alternate cyclic passage with a mosquito vector Yet only one o

246 metachirus captured in the area showed immunity. There w higher percentage of positive neutralization tests (average 7 per cent) the sera of the several species of the genus *Marmosa* that were captured the specificity of some of the neutralization tests on these animals was q tionable.

Bugher, Boshell-Manrique, et al. (1941) found that of 55 specimens *Didelphis marsupialis* captured in a locality in Colombia where yellow fe virus was isolated from mosquitoes, 15 were immune and 4 more becam immune following capture. Because no primates were observed in the fores it was concluded that the didelphis was serving as the vertebrate host. Whi man (1943) also observed some low grade immunity among didelphis trappe in a region following visitation of the forest virus and no immunity among animals of this species deriving from forests through which the epizootic had not passed. Additional data compiled by Bugher on marsupials are pre sented in Table 21.

To quote further from Bugher:

> Of major interest is the situation in Muzo in the Departamento de Boyacá, a locality long famous for its yellow fever. The region is unique for the apparent complete absence of primates other than man. Extended studies through several years have failed to disclose a single primate obtained in the municipio itself. It is therefore a region in which monkeys have no part in the cycle although there is a high endemicity. The investigations have shown that active yellow fever appears to be always present within the general area even in the years when the Viscerotomy Service has failed to obtain positive liver specimens. This appears to be in contrast with the picture in the llanos of eastern Colombia where yellow fever in man appears in waves often separated in time by several years. It was clearly shown by Kerr and Patiño Camargo (1933) that the existence of yellow fever in Muzo is not related to the persistence in other parts of the country and that it is truly endemic there.
>
> The only animals susceptible to yellow fever virus and which are present in large numbers are the marsupials. *Didelphis marsupialis* and *Metachirus nudicaudatus* occur as large populations, *Metachirus* being relatively more abundant than in the llanos. The only other susceptible animal of the region is also the only primate, man himself. It seems likely that the human population plays a part in the epidemiology with some assistance from the marsupials.

On the other hand, Laemmert, de Castro Ferreira, and Taylor (1946) failed to find immunity in any of 227 didelphis captured in an endemic area

TABLE 21

RESULTS OF IMMUNITY TESTS ON CAPTURED MARSUPIALS IN RESTREPO-VILLAVICENCIO REGION, COLOMBIA, IN POSTEPIDEMIC AND INTEREPIDEMIC PERIODS

Marsupials tested	No pos	No neg	Total	Per cent pos
Postepidemic period, 1936–1937				
Didelphis	13	26	39	33
Metachirus	3	6	9	33
Total 1936–1937	16	32	48	Aver 33
Interepidemic period 1938–1939				
Didelphis	7 *	77	84	8 3
Metachirus	0	4	4	0
Caluromys	2	1	3	67
Marmosa	1	0	1	100
Total 1938–1939	10	82	92	Aver 11
Postepidemic period, 1940				
Didelphis	36	121	157	23
Metachirus	2	29	31	6
Caluromys	0	1	1	0
Total 1940	38	151	189	Aver 20

Source Bugher
* All positives were old animals Sera of young animals gave only negative results.

where there was immunity among primates and the virus was isolated from both mosquitoes and marmosets Moreover in the opinion of the writer the experimental evidence on the susceptibility of didelphis in an epidemiologic sense is not convincing nor does the finding of naturally immune specimens necessarily incriminate it as participating in the cyclic passage of the virus in nature It may represent only a dead end infection This likewise applies to the woolly opossum (*Caluromys laniger*) The crucial evidence of the involvement of marsupials would seem to hinge upon whether or not primates though not observed, were actually absent in the forests of the municipio of Muzo

Most of the *Rodentia* can be eliminated on the basis of resistance to extraneural infection with the virus. The ones found moderately susceptible do not fit into the ecologic picture. They are ground animals, and while present in moderate numbers in some forests where yellow fever virus occurs, they are absent in others. However, 17 of 47, or 36 per cent, of dasyprocta captured in a locality in Colombia were found immune. *Potos flavus*, the only species of *Carnivora* showing moderate susceptibility, is a relatively rare animal of nocturnal habits. One of four specimens examined in Colombia showed immunity, but none of 17 specimens captured in an endemic region in Brazil was immune (Laemmert, de Castro Ferreira, and Taylor, 1946). Finally, the nine-banded armadillo, though it can be infected, is apparently incapable of conveying the virus to mosquito vectors. Also, it is a ground animal, not a satisfactory prey for mosquitoes of any sort, and is probably rarely if ever bitten by haemagogus mosquitoes. Thus, according to present knowledge, only the primates and possibly in some regions the marsupials may serve as natural hosts of the virus in the forest cycle.

MASS BEHAVIOR AND GEOGRAPHIC DISTRIBUTION OF FOREST CYCLE IN SOUTH AMERICA

Information on the behavior of forest yellow fever in South America, though far from complete, is more adequate than that on Africa because of the viscerotomy posts and the active yellow fever control services, which in South America exercise a constant vigilance over the occurrence of human infections. Also, with the eradication of the urban vector throughout virtually the entire region where the forest cycle occurs, it can be assumed that any human infections are contracted by forest contact. Inquiry will usually reveal the forest from which the infection derived. Although viscerotomy posts have in the past supplied more information about the current geographic distribution of the virus than any other source, it should be recognized that they furnish only a crude sampling. There is reason to believe that in many epidemic outbreaks the mortality rate among the infected does not exceed 5 per cent. If liver specimens were secured from all of the deceased, this method would reveal only one in 20 of the infected. The proportion of specimens obtained from those who do die of the disease varies greatly with local circumstances. It depends largely upon the distribution of the viscerotomy posts, the energy of the viscerotomist, and the attitude of the people. Soper and de Andrade (1933) estimated from the results of an

immunity survey made following an epidemic of yellow fever in a small Brazilian town that there had been 363 infections yet there were only five deaths In an area where the forest cycle is endemic 25 per cent of the rural population in the age group of 15–19 years was found immune and it was calculated that at least a thousand infections must have taken place since viscerotomy posts were established yet only four positive liver specimens were obtained (Taylor and Fonseca da Cunha 1946) It is difficult to make an over all estimate but probably less than 1 per cent of the actual infections *is revealed by this procedure Obviously this indirect method of detecting* the presence of the virus in forests is applicable only where there is an accessible human population living in contact with the forest and where viscerotomy posts exist Furthermore with the increasing vaccination of rural inhabitants particularly in areas where yellow fever has previously appeared or is thought likely to occur the value of the viscerotomy posts for locating the virus is proportionately lessened

Reports of illness simulating yellow fever have at times been useful in locating the disease particularly in places where there was no viscerotomy post But as other infections notably acute hepatitis and infectious jaundice may mimic yellow fever it is always necessary to confirm the diagnosis of yellow fever by securing either liver specimens from the deceased or acute and convalescent blood sera from those recovering

Immunity surveys in both man and primates have also been widely used in South America for delineating the geographic penetrations of the virus Clearly this method is of no avail in vaccinated populations and though very useful in the past it has become less and less applicable with increasing vaccination Immunity in primates has proved to be of particular interest as it has occasionally brought to light zones of virus activity from which no human infections had been reported The two drawbacks are (*a*) the effort involved in securing a significant number of blood specimens and (*b*) the retrospective nature of the information acquired It is not an easy matter to trap or even to shoot some species of South American monkeys It demands a great deal of work on the part of experienced trappers or huntsmen to obtain an adequate number of blood specimens over wide forestial areas Since the infection of an immune monkey may have taken place at any time within the life of the animal the only clue to the period of infection is the age of the monkey The exact age of adult monkeys cannot be accurately *determined The finding of immunity in an immature animal is the only* way of knowing that the virus was recently present but it must be re

membered that immunity in a very young monkey (under 6 months of age) may be inherited

The periodic testing for immunity in captured and recaptured cebus monkeys was successfully carried out in a study area in Brazil Although no virus activity was detected during the period of observation this procedure possesses interesting possibilities (Laemmert Hughes and Causey, 1949) Notwithstanding the handicaps of immunity surveys in monkeys it is the most promising method of the future for detecting virus invasions (Kumm and Laemmert 1950)

By combining and analyzing all of the data relative to current human infections and immunity in both man and monkeys the impression is gained that the forest cycle is manifest in two forms—endemic and epidemic—or it may be more correct to say enzootic and epizootic That is there are zones in which the infection is regionally enzootic and other zones characterized by periodic epizootics

The expression regionally enzootic is intentionally used as the virus is probably present at any one place within this region for only a limited period It would be more correct to designate these regions as ones of continuous epizootics for it is believed the virus is retained by a process of wandering epidemics In the intensive studies made in Brazil and Colombia limited to a restricted locality within the forest it has been possible to isolate the virus during only a relatively short period (Bugher Boshell Manrique et al, 1944 Boshell Manrique and Osorno Mesa 1944 Laemmert de Castro Ferreira and Taylor 1946) The disease appears to burn itself out as the available vertebrate hosts become immunized and the virus passes on by direct extension to other parts of the forest Its behavior may be compared to that of some of the acute infectious diseases such as measles and whooping cough the regional endemicity of which is created by a process of continuous epidemics From these so called enzootic zones a few positive liver specimens are received year after year Serologic surveys of rural inhabitants have demonstrated widespread immunity increasing with age which is what one would expect where an endemic condition prevails (Soper 1937a Smith Bevier and Bugher 1948 Snijders Pollak and Hoekstra 1947) Similarly sampling of primates has shown existence of immunity throughout this area

Outside the area that is usually considered to be the *enzootic zone* epizootic outbreaks occur from time to time These become known by the sudden appearance of human infections Since they initially appear near the

fringe of the enzootic zone they are regarded as extensions from the more permanent enzootic reservoir The remarkable feature of at least some of these epizootic outbreaks if one may judge by the associated human infections is their rapidity of spread The two more notable instances from the standpoint of the area involved occurred in Brazil commencing in 1934 and again 10 years later in 1944 The first was the more severe and extensive but in both instances the virus presumably spread by successive yearly steps for more than 1 500 km During the active season of the infection it appeared to travel at a rate approximating 200 km a month Other features of these epizootic excursions are again judging by human infections (*a*) their limitation to the warm rainy season (*b*) their usual failure to recur at the same place for more than one year and (*c*) their extension and reappearance further on with the advent of rain and warm weather In their migratory character they simulate on a grand scale the wandering epizootics in the enzootic zone But they differ in that the virus is unable to maintain itself permanently in the newly invaded territory and as far as can be judged completely disappears for an extended period

The reason for believing the virus disappears is based principally upon the absence of recognized human infections during the interim of epidemics Also it has not been possible to demonstrate that monkeys become immune except during the period of the epidemics (Laemmert Hughes and Causey 1949)

As previously mentioned these epizootic excursions have been signalized and their course followed by human infections The map (Fig 52) prepared by Kerr illustrates the year to year sequence of human cases occurring during the epizootic of 1934–1940 in Brazil The first human infections to be diagnosed appeared in the neighborhood of Coronel Ponce in the State of Mato Grosso which is situated on the watershed between the Amazon and Paraná basins The following season the State of Goiás and the western portion of Minas Gerais were invaded and during succeeding seasons the disease spread fanlike as indicated by the arrows on the map to invade virtually all of southern Brazil The arrow to the west shows the probable southern course of the initial outbreak at Coronel Ponce The arrow connecting the epizootics in Mato Grosso and Goiás is put in by Taylor because in 1944–1945 the same sequence of events transpired which leads one to believe that the two outbreaks were related It is possible however that the infection in Goiás was a direct extension from the tributaries of the Amazon which are connected by gallery forests with those of the upper Paraná The eastern

arm of the epidemic as it swung northward after reaching the coast halted short of Espírito Santo probably because it encountered territory rendered noninfectible by previous invasions southward from the enzootic zone around Ilheus. The second epizootic also started near Coronel Ponce and during the first and second season followed with singular fidelity the

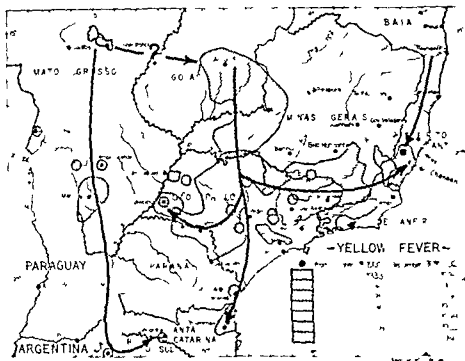


Fig. 52. Probable route of spread of yellow fever epizootic of 1934-1940 in Brazil as indicated by chronologic appearance of jungle yellow fever.

course of the preceding epizootic but it was apparently arrested in its journey southward and eastward from Goiás, as no human infections came to light and immunity tests on monkeys south of the previous season's extension showed no evidence of recent infection. However, in 1938 two deaths from yellow fever did occur far to the southwest—one in the State of Rio Grande do Sul and one in Misiones Territory in northern Argentina. It may be postulated that these cases resulted from a southern extension of the epizootic along the forests flanking the Paraguay and Paraná Rivers from the original "spill-over" at Coronel Ponce. Much of this wooded territory of the

Parana basin is sparsely populated and human infections en route may have escaped notice These epizootics as well as others will be discussed from the human aspect under sylvan or jungle yellow fever

Besides these wide epizootic extensions many minor ones of less extent have been observed about the borders of the enzootic zone particularly in Bolivia Peru and Colombia There are no doubt many epizootic thrusts about the fringe of the enzootic zone where the human population is sparse or has been vaccinated or immunized by previous invasions that escape attention because there are not enough human infections to signal their occurrence As a rule these epizootics are seasonal developing only when conditions are favorable that is during the warm wet period of the year They disappear with the onset of cool or dry weather This seasonal incidence is especially sharp in southern Brazil and Bolivia where the temperature as well as the rainfall is a conditioning factor

They apparently originate from the central reservoir and may be compared to the pseudopods of an ameba protruding outward and then retracting or rather vanishing as the available supply of susceptible hosts is used up They may result from direct extensions in a contiguous forest along rivers or valleys or from a jump over a watershed where the gallery forests on either side come in close proximity The extent and rapidity of spread of these epizootics are dependent upon the size of the area opened to invasion and the prevalence of the vectors and susceptible hosts If the area has not been invaded for a long time and few or none of the monkeys are immune the epizootic will be intense and will spread rapidly At least this is the impression gained from the affliction of the human population in the area An epizootic of this type recently exploded in the Department of Chuquisaca Bolivia where the virus gained entrance to a valley that as far as is known had not been previously invaded at least not for many years The infection presumably spread like a crown forest fire and had engulfed virtually the entire valley by the time it was recognized

It is of interest to quote a communication from George Bevier International Health Division representative in Bolivia

> During the 18 years that a Yellow Fever Service has existed in Bolivia *no* confirmed cases of yellow fever have occurred south of parallel 20°S or in Chuquisaca west of Itimiri and Nacunday which are in turn about 6 km west of Lagunillas and protection tests were uniformly negative south of parallel 20°S The epidemic in Chuquisaca this year [1950] was most severe and most extensive south of parallel 20°S and considerably to the west of the

> places mentioned This new area into which yellow fever extended in 1950 apparently for the first time may really be an epidemic area since some of the older people recall an epidemic having similar symptomatology about 38 years ago which almost wiped out some of the Indian communities and decimated the entire population The high mortality at that time particularly among the Indians who are in constant contact with the forest suggests that they had no immunity and had had no experience with the disease for a long time previously
>
> The epidemic in Chuquisaca this year was severe massive of short duration and affected a considerable proportion of the population clinically and probably a much larger proportion subclinically These facts indicate clearly the absence of immunity in the population and hence the absence of previous outbreaks in recent years The entire area was affected almost simultaneously and no progress of the disease from one region to another could be traced Although a case seems to have occurred on the Rio Azero in October 1949 and there were cases in other parts during the latter part of December the main infection occurred only from mid January to mid April 1950

The interval between the epizootics is unpredictable The second major epizootic in Brazil took place exactly 10 years after the first but as far as *is known there had not been a previous one for more than 20 years* It would seem that the frequency is dependent upon a number of factors that vary in different sectors These factors will be discussed later

Reference has been confined to epizootics about the edge of the enzootic zone but similar epizootics occur within the zone This so called enzootic zone is by no means homogeneous and the only reason for attempting to define it is to delimit the geographic expanse within which the forest virus may permanently exist and from which epizootic excursions may derive There are large areas within the enzootic zone not suitable for the permanent retention of the virus It is probably confined to the larger rain forest type of sylvan vegetation and from these more permanent reservoirs periodic invasions into forests less suitable for permanent maintenance of the virus periodically develop Such epizootics well within the enzootic zone have been noted and studied in Colombia Of what is happening within the inaccessible interior of the vast Amazon Orinoco forests we have no knowledge except that the comparatively few blood specimens that have been collected from docile Indians and from monkeys living in these forests indicate that the virus is widespread

It may be gathered from what has been said that it is exceedingly difficult

to differentiate between the enzootic and epizootic zones first because these differences are to a considerable extent a matter of degree and second because information is inadequate Certain general statements however may be made Perhaps it would be best to refer first to the official map prepared by the International Quarantine Commission demarcating what was then called the endemic region (United Nations Relief and Rehabilitation Administration Epidemiological Bulletin 1946) Minor alterations in the original zone were subsequently made and lately the Yellow Fever Panel of the World Health Organization (1950) modified the terminology to enzootic and epizootic yellow fever areas Fig 53 shows the enzootic areas as designated by the World Health Organization and in addition the farthest points beyond these areas where yellow fever in man has resulted from forest contact or where immune monkeys have been found This latter area for want of a better term may be called the epizootic zone in that it is subject to periodic invasions of the virus but between the invasions the virus presumably disappears It is not unlikely that this area will be extended when more data are accumulated For instance no immunity surveys have been made of the rural populations or of monkeys in Paraguay and Argentina adjacent to Bolivia For the application of international control measures the zoning of the World Health Organization may be adequate but no one would contend that it is accurate Diseases above all those of wild animals do not conform to meridian or political boundaries

The recent recognition in Panama of yellow fever contracted by forest contact has focused attention upon this region (Courtney 1950) The first overall survey for human immunity including Central America and Mexico was published by Sawyer Bauer and Whitman (1937) Nearly all the immunes were found among older people who were living when aegypti transmitted yellow fever was known to exist There were four positive sera three from Mexico and one from San Salvador obtained from children under 10 years of age which suggested that yellow fever had persisted after the last recorded urban epidemic Nevertheless the authors concluded

> Whether the disease may be present in some limited rural areas in the form of the so called jungle yellow fever recently reported from Brazil and Colombia is impossible to determine definitely from the results of our survey But the absence of immunity in children born within the last ten years as well as the absence of recognized cases from both urban and rural communities during this period would seem to render the existence of this type of yellow fever in Mexico and Central America highly improbable



Fig 53 Showing in crosshatch the enzootic yellow fever zone of South America as defined by the Yellow Fever Panel of the World Health Organization and in single line shading the epizootic zone or the farthest points beyond the enzootic zone where jungle yellow fever has occurred or immune monkeys have been found Also included in the enzootic zone is a small area on the coast centered at Ilheus in the State of Bahia

It should be remembered however that most of the sera were collected from urban communities. Also the necessity of considering that immunity in age groups above 15 years may have resulted from infection during the time of urban epidemics eliminates persons who would more likely have contact with forests and thus contract the disease from this latter source. More convincing negative evidence concerning the Republic of Panama was presented by Kumm and Crawford (1943). Their blood collections were made especially from rural populations. They failed to find indication of yellow fever infection west of the Canal Zone, but did conclude that the disease was endemic within 50 miles to the east. In the report by Courtney referred to above seven known deaths from yellow fever are recorded. All were among rural inhabitants who had had contact with the jungles. Immunity tests on blood samples from 100 monkeys collected both east and west of the Canal Zone showed 30 of them to be immune. Further collections by H. C. Clark, director of the Gorgas Memorial Laboratory, are now in progress. Likewise investigations are being pursued on the probable vectors. However it is already evident that the forest cycle of the disease has crossed the canal, which could scarcely be regarded as an effective barrier, and has invaded western Panama. Whether or not this represents a temporary excursion of the virus from Colombia and eastern Panama or is a flare up from pre existing enzootic foci further west and north is not clear and can be determined only by further investigations.

Boshell Manrique (1948) has placed the main reservoir of the virus in the great rain forests of the Amazon Orinoco hydrographic system and traced a connection between this fundamental reservoir and other areas now considered endemic, namely the Ilhéus focus in Brazil, the Magdalena basin, the contiguous forests over the divide in Venezuela and the Guianas, and presumably the extension into Panama.

THE PROBABLE MECHANISM OF THE FOREST CYCLE IN SOUTH AMERICA

The insects that best conform to the criteria of vector efficiency are mosquitoes of the genus *Haemagogus*, and the animals that best qualify as hosts are the primates, with the possible inclusion of some species of marsupials. In addition to experimental evidence and the isolation of yellow fever virus from captured haemagogus mosquitoes by Shannon, Whitman and Franca (1938), there have been published the results of three field studies

which support this view These intensive field investigations we
ducted in areas where the virus of yellow fever was proved to be
during the course of the observations Two were made in Co
(Bugher Boshell Manrique et al 1944 Boshell Manrique and C
Mesa 1944) and one in Brazil (Taylor and Fonseca da Cunha 1946
mert de Castro Ferreira and Taylor 1946) Colombian studies emph
the role of the genus *Haemagogus* in the transmission of the virus and c
attention to some of its behavior characteristics In Colombia *H spegaz*
falco is the most common species of this genus During the two Colomb
studies the virus was isolated from this mosquito on 17 occasions and fr
A leucocelaenus on two occasions Not only was the virus isolated mo
frequently from haemagogus mosquitoes but they were found to be by f
the most common species among the mosquitoes capable of transmitting th
virus To quote from some of the conclusions of Bugher Boshell Manrique
et al (1944)

> The animal phase [of yellow fever virus in South America] is exclusively mammalian and for practical purposes is confined to the primates and the marsupials both of which are arboreal While in these experiments virus was demonstrated in *A leucocelaenus* it appears that the essential vector is *H spegazzinii falco* (revised nomenclature) which is the only species of this genus which has been distinguished in this portion of Colombia Transmission of virus from animal to animal and its persistence in an area are necessarily closely linked with the habits of the vector *H spegazzinii falco* As shown in the course of these investigations this mosquito is outstandingly arboreal and especially during the dry season it may be found only in the tree tops Here it may persist throughout the dry season and infected mosquitoes may thus carry over virus into the next breeding season at the beginning of the rains Then the cycle can be re established and the temporarily suspended outbreak continued For this reason there is an association between human cases of jungle yellow fever and the mid portion of the jungle rainy season

It may be added however that in this region the dry season does not as a rule last more than 2 months and the maximum duration is 4 months and even during this period occasional rains may occur The final conclusions were

> Jungle yellow fever may be sustained among purely monkey or purely marsupial populations or mixtures of the two There is no mammalian reservoir of yellow fever virus virus may persist for the lifetime of the m

that the true reservoir may be said to be in the mosquito vector and not in the mammalian part of the cycle

The studies made in Brazil confirmed the impression of the Colombian investigators that haemagogus mosquitoes constitute the important vectors In the Ilheus region referred to above the species identified was *H spegazzinii* of which *H spegazzinii falco* is considered to be a subspecies In this instance *A leucocelaenus* as well as *A scapularis* could be ruled out as probable vectors in view of their scarcity On the other hand the haemagogus were quite numerous (9 119 of a total of 172 873 mosquitoes captured) The virus was isolated from haemagogus and in the study of different groups of forests there was shown to be a correlation between the prevalence of haemagogus the immunity rate in primates and the immunity rate in persons having contact with the forest The climactic type of rain forest appeared to be the most favorable environment for the permanent maintenance of the virus as it was in these forests that haemagogus mosquitoes were in greatest abundance and that the highest immunity rates in primates and in the human population having contact with the forests were observed It was during these studies that the virus was isolated on four occasions from marmosets the predominating primate of the region Contrary to the observations in Colombia there was no evidence that marsupials particularly didelphis and metachirus were involved in the natural cycle of the virus Besides these published studies many other miscellaneous observations have confirmed the belief that haemagogus mosquitoes and primates are the important vectors and hosts of the virus

The hypothesis of the monkey—mosquito cycle will now be examined with the purpose of deciding whether it satisfactorily explains what has been learned of the epidemiologic behavior of the forest cycle in South America The environmental characteristics of the enzootic zone are the presence of large forests of the tropical rain forest type temperatures that fluctuate within relatively narrow limits and a fairly abundant and well distributed rainfall throughout the year That is the temperature does not fall below the level required for the more or less continuous activity of haemagogus mosquitoes nor is the dry season though it may curtail breeding sufficiently long to prevent the survival of these mosquitoes in considerable numbers Thus the vector reservoir is not threatened and though the disease may become quiescent it is able to continue and progress in the direction of available susceptible monkeys Obviously there must be

gh of the latter as well as of the vectors to keep the chain intact The sity of an adequate supply of nonimmune monkeys would seem to int for the observed shifting of the virus and its failure to remain at d place for a prolonged period With the immunization or death of ionkeys the supply of animal hosts becomes inadequate and in order rvive the virus must pass on to new territory Though there is little nce on the frequency with which the virus retraces its steps beyond fragmentary observations on epizootics outside the endemic zone and poradic recognition of human infections within the zone it appears to ire several years for a locality to become suitable for a new invasion is assumption is correct revisitation of the virus would correspond the period required for replenishment of monkeys through repro on Marsupials reproduce more rapidly and if they served as hosts frequent return visits of the virus would be expected

has been mentioned the favored habitat of both haemagogus mos oes and monkeys is in or near the forest canopy Also the most active ng period (midday) of haemagogus and the resting or napping period onkeys coincide—a circumstance favoring transmission of the virus It erefore logical to surmise that the drama of the forest cycle takes place ily in the forest canopy (Bates 1946) This view is encouraged by the uency of yellow fever infection among woodcutters as the felling of brings down haemagogus mosquitoes which normally remain in the ops

is no strain upon the imagination to explain the spread of the epi ics within the enzootic zone in South America by the normal ranging ands of monkeys and the flight and drift of mosquitoes Monkeys have observed to cross open grassland and shrub from one forest patch to her and may range within the forest for a kilometer or more but their ements are comparatively limited (Gilmore 1943 Bugher Boshell nrique et al 1944 Causey Laemmert and Hayes 1948) It is more bable as will be discussed later that the spread even within contiguous sts and particularly from one forest patch to another takes place by ns of the mosquito vector

he explanation offered for the spread of epizootics within the enzootic e would also apply to the limited excursions of the virus about the phery of the zone The two extensive epizootics that invaded southern zil however present a more difficult problem because of (*a*) the rapid of spread and the distance covered by these epidemics and (*b*) the per

southern Brazil was recaptured 11 5 km from the point of release *Yellow*
A leucocelaenus, another efficient vector was recovered up to 5 7
(Causey Kumm and Laemmert 1950) The region where these ex
ments were carried out was invaded by the epizootic of 1934–1940 an
characterized by scattered patches of forest separated by open grassl
This territory is similar to a considerable part of the epizootic zone
southern Brazil

How should these observations be interpreted? Certainly the experimen on the drift range of mosquitoes give a very satisfactory explanation of th transport of the virus across open country from one forest group to another and it is quite conceivable that by this process the virus can spread over great distances by successive steps Also the observed fact that with a favor able wind haemagogus mosquitoes may travel as far as 11 5 km implies that the spread may be fairly rapid But considering the dilution factor and the time required for replenishing infected mosquitoes at a new point it is necessary to postulate a drift of mosquitoes for distances much greater than 11 5 km to account for the rapidity of progress of the infection as measured by the appearance of yellow fever in humans It was estimated that the epidemic wave of 1944 and 1945 traveled at a rate approximating 200 km per month On the basis of this estimate it would have required 17 flight jumps of 11 5 km within a month to have achieved this distance This would allow less than 2 days at each point to infect monkeys and to pass the infection on to a new supply of mosquitoes That this could occur seems quite unreasonable In fact it would most likely require a second cycle of the virus at each place to build up a sufficient number of infected mosquitoes for the infection to be transported further on That is the few infected mosquitoes wafted to a new forest are not likely to infect more than one to several monkeys and a second generation of the virus in mon keys would probably be required to infect enough vectors to convey the virus to a distant place This second cycle would demand from 12 to 14 days

It is therefore believed that the only way mosquitoes could transport the infection as rapidly as it apparently spreads is by wind currents carrying hem for distances of 50 km or more One would have to assume that on ccasions considerable numbers of haemagogus mosquitoes are picked up om the crown of the forest by an upward draft and being caught in an pper stratum wind current are carried along to be deposited in some tant forest This newly infected forest would constitute a focus from ich neighboring woods could be readily invaded by short range flights

or drifts of mosquitoes, as observed by Causey and Kumm (1948) and by Causey, Kumm, and Laemmert (1950) Such an hypothesis would not only be in keeping with the observed monthly or seasonal spread of the infection, but also with its tendency to proceed irregularly as if by a hurdling process rather than in a regularly progressive manner At least this is the impression gained from the chronologic appearance of human infections Admittedly, this means of measuring the current progress of an epizootic of yellow fever is crude but it is the only one available

We now come to the other puzzling question concerning these extensive epizootics namely, the survival of the virus through the colder dry season The human infections associated with these epizootics occur almost exclusively during the period from December to June, with a peak in January or February That is they begin to appear about two months after the onset of the wet season and they continue to occur in diminishing numbers for about two months after the rains have largely ceased With the return of the wet season human infections again appear, usually further on in the path of the epizootic Such was the sequence of events during the epidemic of 1934–1940 This indicated that the virus though inactive in humans persisted through the colder dry season Since its sojourn in monkeys is transient, it must be assumed that it persists mainly in the vector reservoir

The study made by Causey and dos Santos (1949) on the seasonal prevalence of three suspect vectors is of importance in connection with the survival of the virus (Table 22)

TABLE 22

SEASONAL PREVALENCE OF THREE SUSPECTED VECTORS OF YELLOW FEVER IN BRAZIL

Vector	*Year*	*Season*											
		Wet				*Dry*				*Wet*			
		Jan	*Feb*	*Mar*	*April*	*May*	*June*	*July*	*Aug*	*Sept*	*Oct*	*Nov*	*Dec*
Haemagogus spegazzinii	1945									57	139	600	961
	1946	1,687	456	327	254	58	2	1	4	0	100	849	539
	1947	383	421	403	400	12	1	0	2	34	211	233	254
Aedes leucocelaenus	1945									40	62	236	216
	1946	419	83	8	63	15	2	6	15	3	197	537	146
	1947	291	500	215	129	13	4	2	14	162	190	193	131
Aedes scapularis	1945									5	79	647	561
	1946	31	5	2	16	4	18	60	26	6	28	85	6
	1947	198	477	123	94	56	36	89	93	180	23	137	146

Source Causey and dos Santos 1949

It is notable that during 3 to 4 months in the dry season the two proved natural vectors *H spegazzinii* and *A leucocelaenus* were captured in very small numbers Somewhat larger numbers of *A scapularis* were taken during this period but as heretofore noted the ecology of this mosquito is not well adapted to the monkey–mosquito cycle As only mosquitoes attempting to secure a blood meal were captured these figures may not give a true index of the mosquitoes actually present However for transmission of the virus the mosquitoes must feed and the few attempting to do so implies that little or no transmission occurs during the dry cool season The other alternative is that a few infected mosquitoes remain alive though relatively inactive through the dry cool season to initiate the cycle with the return of warm weather Since proof is lacking it is a matter of opinion whether or not the survival of the virus can be explained in this manner This likewise applies to the explanation offered for the rapidity of spread of the infection by the drift of mosquitoes But before rejecting these hypotheses it is well to remember that no cycle other than the mammal–mosquito one has been disclosed and that the retention of the virus in the epizootic zones from one season to another hangs by a rather fragile thread as it is rarely prolonged beyond one or two seasons within a wide range of territory

It has been suggested as an alternate hypothesis that fledgling birds may be susceptible and the virus may be transmitted by some avian ectoparasite Kerr and de Castro Ferreira did considerable work with pupiparous flies but were unable to demonstrate transmission Interest in avian ectoparasites was subsequently revived by the isolation of equine and St Louis encephalitis viruses from bird mites and by the discovery that St Louis encephalitis virus underwent transovarian passage

Ectoparasites from 162 birds and from 230 bird nests were collected in an endemic area in Brazil where a human infection had recently occurred and they were examined for virus with negative results These limited negative findings however are of no great significance particularly as ectoparasites were found in only 30 of the nests examined

THE PROBABLE FOREST VECTORS IN AFRICA

The African mosquitoes shown by laboratory experiment to be capable of transmitting yellow fever virus have been listed and described in Chapter 5 and reference was made to some of them in discussing the man–

mosquito cycle Though a number of these mosquitoes are found within forests or about the edges there is only one that has been conclusively incriminated in the forest cycle namely *A africanus* This mosquito is widely distributed throughout the forests of central Africa and like mosquitoes of the genus *Haemagogus* in South America it is a tree hole breeder and is found principally in the forest canopy Also like the haemagogus mosquitoes it may descend to the ground level particularly along the forest edges It differs from the haemagogus however in that it feeds after sunset rather than during the day It is partial to monkeys as a source of its blood meals (Haddow and Dick 1948) It is a good experimental transmitter and virus has been isolated from wild caught specimens in East Africa on five occasions (Haddow Smithburn et al 1948 Smithburn Haddow and Lumsden 1949) The evidence proving this mosquito to be the important forest vector in Uganda will be referred to later

T africanus has been suggested as a possible vector but has not been definitely incriminated (Haddow Smithburn et al 1948 Mahaffy 1949) *A simpsoni* should also be included here as it may be infected from monkeys although it probably plays an insignificant part in the deep forest cycle Its importance lies in its role as an intermediate conveyer of the virus from the forest to man and will therefore be discussed under jungle yellow fever

Finally it should be mentioned that Smithburn Haddow and Lumsden (1949) presumably isolated yellow fever virus under rather peculiar circumstances from a suspension of *Phlebotomus* spp captured during the epizootic episode when the virus was repeatedly obtained from sentinel monkeys and *A africanus* However many arthropods may retain the virus for a short period though unable to transmit it by bite and finding the virus in the body of an insect does not in itself prove the insect is a vector It must be coupled with evidence of ability to transmit Such evidence is lacking for phlebotomus moreover what is known of the ecology of sylvan species of this insect does not point to them as vectors

THE PROBABLE FOREST HOSTS IN AFRICA

Like the South American primates the African primates including the suborder *Lemuroidea* are generally epidemiologically susceptible to yellow fever virus They may be infected by the bite of mosquitoes or by small doses of the virus administered extraneurally and following this the virus

It would be presumptuous to attempt to outline the geographic d
tion of the forest virus in Africa Information is too meager Here i
trast to South America the disease in humans is not a reliable ind
over large parts of Africa domestic vectors exist and it is therefore i
sible to differentiate between yellow fever contracted from the forest
that resulting from the man–mosquito cycle The only accurate metho
identifying the forest cycle is through determination of naturally acqu
immunity in monkeys Immunity surveys in forest primates though int
sively conducted in Uganda on the east coast and to a considerable deg
in Nigeria and the Cameroons on the west coast are not extensive in ge
graphic scope Elsewhere insufficient sampling has been done Howeve
positive sera from monkeys have been reported on the west coast as far north
as Senegal and as far south as the Belgian Congo (Findlay Stefanopoulo
et al 1936 Findlay and MacCallum 1937*c* van den Berghe 1939*b*
Durieux Boiron and Koerber 1946) In East Africa Findlay and Mac
Callum (1937*c*) found one positive blood in 19 specimens deriving from the
Anglo Egyptian Sudan and a very high percentage of the monkeys of the
lowland rain forests of Uganda are immune (Haddow Smithburn et al
1947) In central Kenya and in the Gede forest near the Indian Ocean
Smithburn and Haddow (1949) found immune monkeys and galagos

Certainly there is ample proof that the forest cycle is endemic in low
land forests of Uganda and for lack of better information it may be in
ferred that it is likewise endemic in other African forests that furnish
a similar biologic environment In connection with this criterion as a
basis for outlining the enzootic zone the following quotation from Haddow
(1945*a*) is of interest

The first real attempt to work out the zoogeography of the Ethiopian Region
(i e Africa south of the Sahara and part of Arabia) was made by Chapin
in his classic paper on birds (1923) With small modifications Chapin s work
has been found to apply with equal truth to the distribution of other forms of
life Under this system the Ethiopian Region is divided into two subregions
The first of these the West African Subregion includes a broad belt along
the Gulf of Guinea almost the whole Congo Basin (except the High
Katanga) and most of Uganda All the rest of the Ethiopian Region goes to
form the East and South African Subregion The great rain forest desig
nated the Guinea Forest Province is divided into 2 districts The first com
prising the coastal forest area round Cape Palmas in the west is referred
to as the Upper Guinea Forest District The second the Lower Guinea

Forest District (Fig. 54) includes the vast forested area of the Congo Basin, with the lowland forests of Uganda as outliers. Geographically Bwamba belongs to the Lower Guinea Forest and faunistically also its western affinities are very clearly marked. It is necessary to stress this point as there is a tendency to regard even the western parts of Uganda as essentially East African.

Figure 54, referred to above, has been modified to show the geographic limits of yellow fever in man revealed so far by immunity surveys. It will

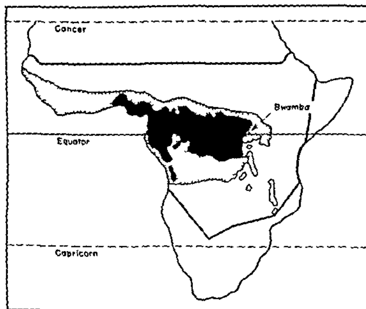


Fig. 54. Ethiopian region showing the West African subregion (stippled) and the Lower Guinea forest district (black). The small lowland forests of Uganda are omitted. The black line connects the farthest points where immunity in man has been found.

be seen that the disease in man extends northward, southward, and eastward far beyond the territory covered by the Lower Guinea forest district and the West African subregion. The question arises: are these extensions of the disease in man connected with the forest cycle or do they derive from endemic foci of the man–mosquito cycle? This question cannot be answered at present. It will require a careful investigation of the epidemiology of the human disease as well as extensive immunity surveys in forest

primates before an opinion can be expressed It may be remarked, that the relatively low and scattered immunity found in Kenya, Tan Northern Rhodesia, and as far south as northern Bechuanaland is r one would expect to find as a result of either epidemic or endemic mosquito cycle yellow fever.

PROBABLE MECHANISM OF THE FOREST CYCLE IN AFR

Virtually all information concerning the mechanism of the forest in Africa stems from studies made in Bwamba County, Uganda Bwa County is situated in the extreme west of Uganda and borders on the gian Congo There are three main areas within the county. one compr mountain slopes and may be classed as temperate rain forest and mount grassland, the second consists of the acacia–tall grass savannah of the Se liki Plains, and the third contains the main lowland forests of the tropica rain forest type It was in the third area that nearly all of the epidemiologi and zoologic work was conducted (For more detailed description see Had dow, 1945*a* and *b*) This area was selected for study because surveys of the native inhabitants revealed a higher rate of immunity than had been found elsewhere in the county (Hughes, Jacobs, and Burke, 1941, Mahaffy, Smith burn et al, 1942) Although the area was kept under close observation for a period of 2 years (1937–1939), no clinical yellow fever was observed, and attempts to isolate the virus from humans and mosquitoes were unsuccessful Nevertheless, a resurvey made in 1941 revealed that 48 individuals among 168 who were nonimmune when tested a year to a year and a half previously had become immune (Mahaffy, Smithburn, et al, 1942) As a result of this finding, which clearly showed that the virus was being transmitted to the human population, the work was intensified both with regard to sur veillance over the human inhabitants near the forest and to the study of the animal and insect fauna of the forests

This increased effort was rewarded by the isolation of yellow fever virus from a native woman and from mosquitoes of the species *A simpsoni* cap tured in the vicinity of houses (Mahaffy, Smithburn, et al, 1942) It will be recalled that in this region *A. simpsoni* breeds in plant axils and is preva lent about houses and along the forest edge (Haddow, 1945*b* and *c*, 1948) The ecology of the mosquito is well adapted to the introduction of the virus from the forest and to transmission of the disease among village resi dents after the virus is introduced But since it is not found to any extent in the deep forest, it could scarcely be responsible for transmitting the disease

from monkey to monkey except near the forest edge and in the vicinity of banana and other types of plantations, which in Uganda constitute the principal place of breeding of this mosquito As it was demonstrated that a high rate of immunity existed among monkeys far from human habitations, it was necessary to postulate another vector (Haddow, Smithburn, et al, 1947) A series of careful entomologic surveys showed that *A africanus* was the most prevalent sylvan mosquito capable of transmitting the virus experimentally (Haddow, Gillett and Highton, 1947, Haddow and Mahaffy, 1949)

Although a large number of mosquitoes were tested for virus, no further isolations were made until some years later (Smithburn and Haddow, 1946) In the meantime, there had been an effective mass immunization of the human population, and it seemed impossible that the disease could have remained endemic by means of the man–mosquito cycle Nevertheless, the virus was again obtained from *A simpsoni* captured near houses and also from a batch of aedes mosquitoes taken in a part of the forest far removed from human dwellings *A africanus* was among the aedes mosquitoes tested and was suspected of being the species harboring the virus At least this observation showed that the virus was present in aedes mosquitoes other than *A simpsoni* captured in the deep forest The final and conclusive evidence incriminating *A africanus* was obtained later (Haddow, Smithburn, et al, 1948, Smithburn Haddow and Lumsden, 1949) During a period of 5 months, eight infections of sentinel monkeys were observed, and in addition the virus was isolated on four occasions from *A africanus* captured in the vicinity of the infected sentinel monkeys The monkeys were stationed along a trail running through the Semliki forests for a distance of approximately 14 miles This forest area was far from any human habitations, thus eliminating all possibility that the virus might have derived from a human source The number of *A africanus* examined for virus was 5,258 It is of particular interest that all the infected sentinel monkeys and *A africanus* were found in a 2⅓ mile section of the trail which suggests the localized character of the virus invasion

The points in favor of *A africanus* as the principal vector in the forest cycle in Uganda have been summarized by Haddow, Smithburn, et al (1948) as follows

1 *A africanus* has a very wide distribution in central Africa
2 It is an efficient vector of yellow fever under laboratory conditions, as has been shown by Philip (1929) and by work in the Yellow Fever Research Institute (Entebbe)

3 It was included in the infected lot of *Aedes* spp taken in 1941

4 It is the dominant culicine of the forest canopy in Bwamba

5 Catches made in trees in many parts of Bwamba (and in the E area) have shown only three mosquito species common to every tr every series *A africanus* is one of these One of the others (*A* (*S*) *argenteus* Theo) has proved incapable of transmitting virus u laboratory conditions in West Africa (Bauer, 1928) and at Entebbe third, *Taeniorhynchus* (*Mansonioides*) *africanus* Theo, is known to capable of transmitting yellow fever virus in the laboratory (Phi 1930) but was not included in the infected lot of 1941

6 *A africanus* has been taken in trees in each of the 17 Uganda localities an altitude of under 5 000 feet (in addition to Bwamba and Entebb which have so far been studied

7 It bites after dark This is an important point, as some monkeys, know to be involved in the forest cycle of yellow fever spend much of their time on the ground by day, but by night (in Bwamba, at least) all species sleep in trees

8 It survives dry weather in the adult state in forested and wooded areas in Uganda (Haddow, Gillett, and Highton 1947), and is therefore capable of carrying over virus from one wet season to the next

9 It readily bites monkeys in captivity (Haddow and Mahaffy, *in press*) and on tree platforms (Haddow and Dick, *in press*).

We know of no other mosquito, with the possible exception of *T africanus*, which fulfills all these conditions

With regard to vertebrate hosts, the high rate of immunity among pri mates (61 per cent), the density of the primate population in the Semliki forests, estimated at 400 per square mile (Haddow, Smithburn, et al, 1947), and the lack of information incriminating any other host make a convinc ingly strong case in favor of primates being the dominant, if not the only, warm blooded hosts The high rate of immunity among strictly arboreal species indicates that transmission occurs above the ground level This is in harmony with the habits of *A africanus* It is presumed, therefore, that the forest cycle of the virus takes place largely in the upper canopy of the forest Here again we find a striking analogy with the situation in South America, the only difference being that the African vector is a crepuscular and night feeder, while in South America the vector feeds during the bright hours of the day In Africa the monkeys are probably infected when they retire for the night's sleep, and in South America they are probably infected

when they rest or nap around midday It may be deduced therefore from these Semliki forest studies that the mechanism of the forest cycle revolves about the mosquito *A africanus* and the forest primates (Mahaffy 1949) It will be recalled that *A africanus* is not found in mountain forests above an altitude of 5 000 feet nor has immunity been detected in monkeys that permanently inhabit the mountain forests above this altitude

The entomologic studies made on the west coast principally in Nigeria have revealed the wide distribution of *A africanus* as well as of immune monkeys in the forests and it may be presumed that the forest cycle is maintained in a like manner and by the same or similar combination of host and vector as in Uganda

However in a more recent publication Haddow Dick et al (1950) have raised the question of the existence of a diurnal vector in order to explain the higher immunity rates among species of monkeys habitually arboreal *than are found among the partially terrestrial species The latter sleep in* the trees and should be equally as exposed at night to *A africanus* as are the exclusively and mainly arboreal species These authors also found difficulty in explaining the occurrence of epizootics among monkeys on islands in Lake Victoria and in isolated patches of forests separated by 10 miles of grassland from the main forests as well as the occurrence of yellow fever in areas with a prolonged dry season They conceded the possibility of the virus being carried for long distances by mosquitoes caught in wind currents and the possibility of its persistence through the dry season in the monkey—mosquito cycle since Robinson (1950) found that females of *A africanus* do survive the 6 month dry season in Northern Rhodesia Nevertheless they believed that the existence of some other still unknown host—vector cycle should be considered To quote from their discussion on this subject

> while the monkey to monkey mosquito borne disease is indubitably important and while it may adequately explain the situation in the vicinity of the larger forests there may well be some other cycle of which as yet *nothing whatever is known This view has persistently come to the fore* even in the face of much negative evidence based on work on many groups of nonprimates and on arthropods other than mosquitoes both in South America and Africa At present we are gradually tending to the view that *the monkey to-monkey cycle is like the man to-man cycle the end point* of some chain of incidents of which we know nothing We do not in any

way wish to deny the importance of the monkey to monkey cycle nor to doubt that it must be the usual starting point for human infection in Uganda We do however feel that in certain areas at least this cycle cannot be a continuous one that the monkey disease must from time to time die out and that there must be some other source of infection from which it renews itself

JUNGLE OR SYLVAN YELLOW FEVER

The term jungle yellow fever is used here to designate the disease in man contracted by the bite of a sylvan mosquito infected with virus derived directly from the forest cycle Many do not fancy the use of the word jungle in this connection as it implies a thick tangled mass of impenetrable vegetation that is not the kind of environment best suited for the propagation of the forest cycle Certainly the interior of tropical and subtropical rain forests with which the virus is commonly associated does not have the characteristics of a jungle It is only about the edge of these forests that the ground vegetation is thick and jungle like The term jungle yellow fever was coined when the most favored environment of the forest virus was not well understood and since human infections are frequently contracted about the forest edge where the ground vegetation may simulate a jungle there was some excuse for its choice In view of subsequently acquired information and for consistency with the Spanish French and Portuguese designations of *sylvestre* and *silvestre,* it would seem preferable to use sylvan or sylvatic in English But jungle yellow fever has priority and has received wide usage It also has a dramatic and fearsome ring and the name will probably stick

IN SOUTH AMERICA

Jungle yellow fever is virtually the only existing epidemiologic form of the disease in the New World The last known case of probable aegypti transmitted yellow fever occurred in one locality in Peru in 1912 Some idea of the relative importance of jungle and aegypti transmitted yellow fever may be obtained from the reports of the yellow fever control services of Brazil Colombia Peru and Bolivia of confirmed cases during the past 15 years (Table 24)

TABLE 74

DEATHS FROM YELLOW FEVER CONFIRMED BY EXAMINATION OF LIVER SPECIMENS, BRAZIL, COLOMBIA, PERU AND BOLIVIA, 1935–1949

Period	*Aedes aegypti* present*							
	Brazil		*Colombia*		*Peru*		*Bolivia*	
	Yes	*No*	*Yes*	*No*	*Yes*	*No*	*Yes*	*No*
1935–1940	27	1 072	0	182	1	1	6	66
1941–1945	3	159	0	153	3	38	0	47
1946–1949	0	11	0	136	1*	14	0	33
Total	30	1 242	0	471	5	53	6	146

* Although a few aegypti were present in the area, it is believed that infection was acquired from forest contact (La rte)

The foregoing figures represent only fatal cases diagnosed by liver examination by viscerotomy services and therefore comprise only a small percentage of the actual infections. But they will serve to indicate the relative frequency of the two epidemiologic forms of the disease. It will be noted that of the 1 953 cases diagnosed by viscerotomy since 1934, 1 912, or 97.9 per cent, occurred in the absence of aegypti.

As jungle yellow fever is a by-product of the forest cycle, what has been said in regard to the geographic distribution and movement of the forest cycle likewise applies to jungle yellow fever. Indeed, as previously explained, the existing information on the occurrence and movement of the forest cycle in South America has been derived largely from associated human infections. A separation can therefore be made into endemic and epidemic zones that correspond with the enzootic and epizootic zones. An indication of the prevalence of the disease in parts of the endemic zone is indicated by the results of immunity surveys. To cite a few examples, the survey published by Soper (1937*a*) showed immunity rates ranging from 45 per cent to 85 per cent among Indians above 15 years of age living in the forested areas of the Amazon Valley in Brazil, and it was calculated from these data that infection was occurring at an annual rate of about 3 per cent (Muench, 1934); similar rates were obtained by Smith, Bevier and Bugher

(1913) in certain rural areas in Colombia more recently a study in an endemic rural area near Ilheus Brazil demonstrated that one quarter of the population in the age group of 15 to 19 years was immune (Taylor and Fonseca da Cunha 1946) While the immune rates vary greatly from district to district these and other sampling surveys illustrate the wide preva

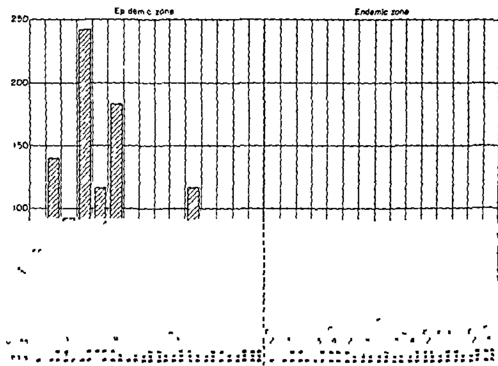


Fig 55 Fatal cases of jungle yellow fever (by year) in the epidemic and endemic zones of Brazil 1935–1949 as diagnosed by examination of liver specimens (*Data from report of the Yellow Fever Service of Brazil 1949*)

lence of jungle yellow fever within the endemic zone where rain forests exist

In Brazil the epidemics extending southward from the endemic zone have produced the greater number of recognized fatalities (Fig 55) This may be attributed primarily to the density of human populations in these areas and their accessibility for study as compared with populations of most of the endemic regions Also there is reason to suspect that the human disease may be more severe and give rise to a greater fatality than commonly occurs in some of the places within the endemic zone Certainly in the neighborhood of Ilheus Brazil judging from the immunity rates the case fatality rate

is very low and the disease smolders along commonly without being clinically recognized On the other hand the explosive character of some of the southern epidemics is dramatic particularly if the human as well as the monkey populations are highly susceptible that is if there has been no recent preceding invasion and the human population has been vaccinated only partially or not at all This was illustrated by the Brazilian epidemic of 1934–1940 during which 1 020 positive liver specimens were received and by the more recent outbreak that occurred in the Department of Chuquisaca in southern Bolivia The course of the epizootic with which the Brazilian epidemic of jungle yellow fever was associated was discussed under the forest cycle of the virus An account of the human infections resulting from this epizootic from 1934 to mid 1938 has been published by Soper (1938*a* and *b*) The Chuquisaca epidemic apparently resulted from the entrance of the virus into a valley that as far as is known had not been invaded previously at least not for many years In a population approximating 17 000 there were an estimated 850 clinical infections with 230 deaths within a period of a few months

Besides the susceptibility of the monkey and human populations there is a third biologic factor which contributes to the dissemination of the virus and the incidence of human infections namely the prevalence of the vector It has been noted in many isolated instances that haemagogus mosquitoes are unusually numerous during these epidemic excursions Bevier writing about the recent epidemic in Bolivia said

> During epidemics of jungle yellow fever it has frequently been noted that haemagogus mosquitoes have multiplied to such an extent as to become a pest entering houses and biting avidly In the Santa Cruz area they frequently are found breeding in domestic barrels and tanks and the larvae at first glance closely resemble *A aegypti* During the 1950 epidemic in the valleys of the Azero and Parapeti Rivers these mosquitoes were present in extraordinary numbers and entered the houses many people showed an effect of biting similar to that which occurs when people from the Altiplano visit the low hot regions and their legs and other exposed parts show evident signs of insect bites At Saucemayu attempts were made to vaccinate in a house on a hill but the wind was so bad that the vaccinating outfits were moved down to the edge of a stream in the open where there was less wind In the house in spite of the wind the haemagogus were exasperatingly abundant and avid to bite but near the river none were seen The rainy season during which this epidemic occurred had been unusually wet and the

roads and trails had suffered more damage than during ordinary rainy seasons

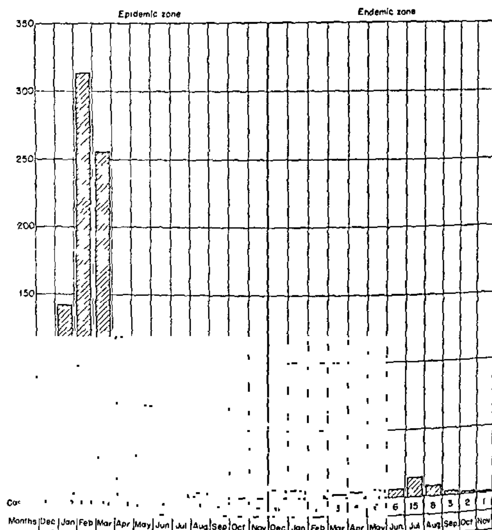


FIG 56 Seasonal incidence (by month) of fatal cases of jungle yellow fever in epidemic and endemic zones of Brazil, 1935–1949, as diagnosed by examination of liver specimens (*Data from report of the Yellow Fever Service of Brazil, 1949*)

However, as will be shown later, if the virus is present in the forest, contact with the forest plays the dominating part in the acquisition of jungle yellow fever

Seasonal Incidence The seasonal incidence of jungle yellow fever in the southern epidemic zone is strikingly different from that within the endemic

zone. This is illustrated by the monthly distribution for the years 1935–1949 of fatal infections diagnosed by the Viscerotomy Service in Brazil (Fig. 56).

In the epidemic zone the disease is limited to the warm wet season, while in the endemic zone cases may occur throughout the year. This is further shown by the seasonal distribution of jungle yellow fever in Colombia, which is within the endemic zone (Table 25). In referring to the endemic zone, what is meant is regional endemicity, as most of the human infections in Colombia are associated with local epidemics. While there is some variation in seasonal incidence within the endemic zone, it is not so marked. It seems quite clear that the determining factor in the seasonal incidence of jungle yellow fever is the prevalence of the vector, as there is a correlation between the prevalence of haemagogus mosquitoes and the incidence of the human disease. This correlation is more striking in the epidemic zone, but it also exists to a lesser degree within the endemic zone (Bugher, Boshell-Manrique, et al., 1944; Gast Galvis and Bates, 1945; Kumm, 1950).

TABLE 25

MONTHLY INCIDENCE OF JUNGLE YELLOW FEVER IN COLOMBIA DIAGNOSED BY THE VISCEROTOMY SERVICE

1934–1949

Months	*Number of cases*	*Monthly percentage*
January	73	15.1
February	29	6.0
March	11	2.3
April	11	2.3
May	27	5.6
June	35	7.2
July	64	13.2
August	37	7.7
September	36	7.5
October	31	6.4
November	52	10.8
December	77	15.9
Total	483	100.0

Forest Contact. After it was conclusively demonstrated that yellow fever may exist in the absence of aegypti, it soon became evident that the disease

was associated with forest contact. A number of instances supporting this conception are cited by Soper (1935*b*, 1936*b*, 1938*a* and *b*). Viewed in retrospect, several of the earlier studies in Colombia going back to Franco, Martínez Santamaría and Toro Villa (1911) and later studies by Kerr and Patiño Camargo (1933) and Boshell Manrique (1938) are clearly indicative of jungle yellow fever. Also, the epidemics reported by Pena Chavarría, Serpa and Bevier (1930) probably arose from the forest virus, and an undetermined number of human infections during these epidemics may have resulted from forest contact. Burke (1937), investigating an epidemic of jungle yellow fever in the State of Mato Grosso, Brazil, concluded that "Well over 90 per cent of the patients whose cases were analyzed gave a history of contact with clearings during the probable incubation period ... and that ... infection generally occurred through contact with the jungle or in close proximity to it as in agricultural clearings." Forest contact was shown to be the dominating factor during the 1934–1940 and 1944–1945 epidemics in Brazil and is likewise emphasized by case histories in Colombia. In an investigation in an endemic region in Brazil the relation of forest contact to immunity was studied. Results are shown in Table 26 (Taylor and Fonseca da Cunha, 1946).

TABLE 26

RELATION OF FOREST CONTACT TO IMMUNITY IN AN ENDEMIC REGION OF BRAZIL

	Visits to						*Neither*	*Both*	*Total*
	Old-type forest only			*Young-type forest only*					
	Daily	*Weekly or monthly*	*Total*	*Daily*	*Weekly or monthly*	*Total*			
Immunes	24	11	35	29	21	50	7	62	154
Total	35	35	0	118	165	283	194	160	707
Per cent immune	68.6	31.4	50.0	24.6	12.7	17.7	3.61	38.8	21.8

Source: Taylor and Fonseca da Cunha, 1946.

The role of forest contact in jungle yellow fever is stressed because it is the basis of other epidemiologic characteristics of the disease, such as occupation, age and sex distribution, and location of habitation.

Relation to Place of Habitation Burke (1937) following the above mentioned epidemic in Mato Grosso collected sera from inhabitants of the region and divided them into three groups Group 1 (91) included sera from urban inhabitants Group 2 (150) and Group 3 (430) consisted of sera from persons living more than 3 km and less than 3 km respectively from fields or jungle Neutralization tests performed on these sera gave the following immunity rates Group 1 7 5 per cent Group 2 16 7 per cent Group 3 39 3 per cent These rates suggest that the location of the dwelling in relation to the forests or clearings therein is of significant importance Taylor and Fonseca da Cunha (1946) found a similar relationship but when corrections were made for the frequency of visits of individuals to the forest the correlation between the proximity of the habitations to the forest became insignificant That is the higher immunity rates among persons living near the forest could be explained by the greater frequency of visits within the forest but among those who did not visit the forests the immunity rate was low irrespective of where they lived For example the ratio of immunes among those who lived within a short distance (500 m) of the forest but who did not visit the forest was no higher than among persons who lived at a greater distance and likewise did not visit such forests It would appear therefore that entry into the forest or working along its edge constitutes the main hazard of contracting the disease However there may be some exceptions to this general rule applying to habitations directly on the forest edge For example Burke recounted finding all of the 11 members of a family of different ages who lived near a forest showing immunity to yellow fever It is conceivable therefore that in unusual circumstances applying particularly to intensive epidemics where *haemagogus* are in abundance these mosquitoes may enter houses near the forests and transmit the infection within the house or about the immediate premises and in this way infect all members of the household The above-quoted communication from Bevier suggests that some transmission of this sort may have taken place during the recent epidemic in Bolivia

Occupation It has been said that jungle yellow fever is an occupational disease This is true if the definition of an occupational disease is extended to include any occupation which tends to bring the individual into contact with the forest Woodcutting the clearing of land for cultivation and the felling of trees for running roads or trails through forests is especially hazardous as such work not only brings the person in contact with the forest but also through the felling of trees brings the haemagogus mosquito down to

the ground level and into more direct contact with man The danger of this type of occupation has been especially emphasized by Boshell Manrique and Osorno Mesa (1944) and by Bugher, Boshell Manrique et al (1944) But the disease is by no means limited to professional woodcutters The Ilhéus study (Taylor and Fonseca da Cunha 1946) revealed the relation of occupations to yellow fever immunity as shown in Table 27

TABLE 27

RELATION OF OCCUPATION TO YELLOW FEVER IMMUNITY, ILHEUS, BRAZIL

Occupation	*Males*		*Females*		*Both sexes*	
	Number tested	*Per cent positive*	*Number tested*	*Per cent positive*	*Number tested*	*Per cent positive*
Laborers cacao plantations	165	47 9	78	25 0	243	40 7
Managers cacao plantations	8	37 5			8	37 5
Woodcutters (charcoal)	7	28 5			7	28 5
Laborers mandioca farms	62	27 4	61	11 5	123	19 5
Domestic	2	50 0	67	10 4	69	11 6
No occupation (children)	136	8 8	115	5 2	251	7 2
Miscellaneous	6				6	
Total	386	29 8	321	12 2	707	Av 21 8

Source Taylor and Fonseca da Cunha, 1946

In this locality the raising of cacao is the important industry The ca plantations though sometimes quite extensive are bordered by for Haemagogus mosquitoes occur within the cacao plantations and marmo which are abundant, range into the cacao vegetation from the edges of forest creating a biologic environment favorable to temporary extensio the virus A high incidence of infection was noted among coffee pickers epidemic of jungle yellow fever in Peru It is apparent therefore, th major occupation most conducive to infection with jungle virus m under different circumstances depending upon whether or not it the person in contact with an environment conducive to the propag the forest virus The field worker s habit of repairing to the sha nearby forest for lunch or rest during the hot hours of the day is one of the more frequent means of his becoming infected

Age and Sex Distribution It will be of interest first to compare the age and sex distribution of jungle yellow fever with that of aegypti transmitted yellow fever This is well shown by data presented by Soper (1937*b*) He divided aegypti transmitted yellow fever into two categories urban and rural The so called urban yellow fever resulted from epidemics within towns

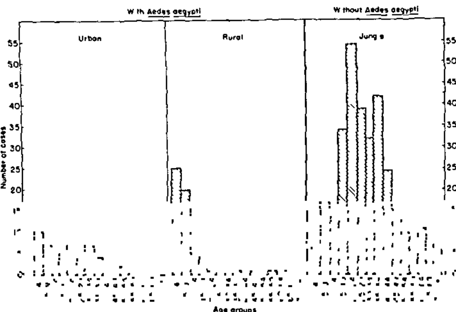


FIG 57 Distribution, by age, of fatal cases of rural urban and jungle yellow fever in Brazil Colombia and Bolivia, confirmed by examination of liver specimens January 1, 1930, through June 30 1936 (*Soper, 1937b*)

while the statistics on rural yellow fever derived from an area where the disease had been endemic for a number of years (Fig 57) Under the endemic condition prevailing in the rural districts, the older age groups had been immunized to a larger extent than they had among the urban population which had not been so continuously subjected to infection Thus, it is found that a higher mortality occurred in the rural endemic region in the low age groups than among those of more advanced age In jungle yellow fever, it will be seen that comparatively few deaths occur among young children and that the greatest number of deaths are in the age groups between 15 and 40 years

Soper (1937*b*) also gave the results of immunity surveys made in several

Brazilian cities where aegypti transmitted yellow fever had occurred and the results of one survey made after a rural epidemic where aegypti were absent (Figs 58 and 59) In Brazilian cities where the disease was transmitted by aegypti the immunity rate among the females equaled or exceeded that among the males In the immunity survey made following the rural epi

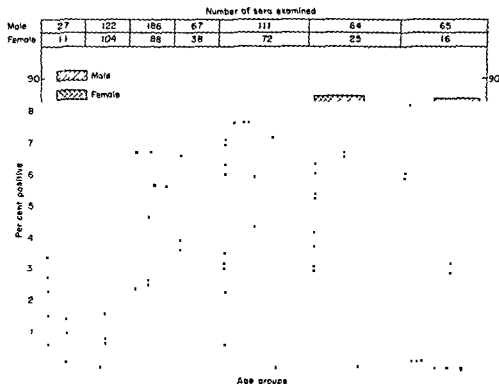


Fig 58 Distribution, by age and sex, of immunity to yellow fever, as shown by mouse protection tests, in a number of Brazilian cities where the disease is transmitted by *Aedes aegypti* (*Soper, 1937b*)

demic in the Valle do Chanaan, where aegypti were absent, immunity among males far exceeded that among females Also, in urban districts with aegypti transmitted yellow fever there was quite a high immunity rate in the lower age groups (15 years and under) but where there were no aegypti the younger age groups showed a very low rate of immunity as compared with that of an older age group

Similar statistics were collected by Taylor and Fonseca da Cunha (1946) in a jungle yellow fever endemic area in Brazil (Fig 60) There was a sud

len rise in the immunity rate at about the fifteenth year of age particularly among males The over all immunity rate among the males was 16 5 per cent in comparison to 16 5 per cent among the females This high rate of immunity among males and in persons above 15 years of age was correlated with visits to the old type of forests in the region For example the males

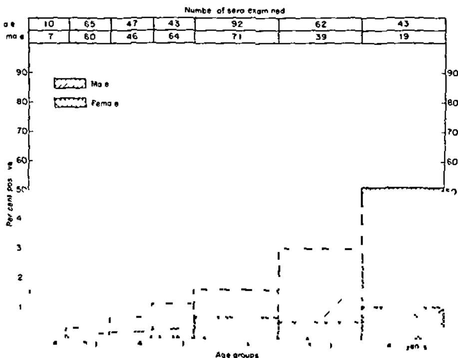


Fig. 59 Distribution by age and sex of immunity to yellow fever as shown by mouse protection tests in the Valle do Chanaan Brazil where the disease is transmitted in the absence of *Aedes aegypti* (*Soper 1937b*)

visited forests about twice as frequently as did the females and persons above 15 years of age visited the forests more than twice as frequently as did those below 15 years Hence the age and sex distribution of jungle yellow fever can be explained largely if not entirely by the relative frequency of forest contact in the different age and sex groups

More massive data supporting this difference in age and sex distribution are shown in Tables 28 and 29 furnished by the viscerotomy services of Brazil and Colombia It will be seen that deaths from jungle yellow fever in males

far outnumber those in females and that relatively few deaths occurred in persons below the age of 14 years Also the peak is reached in the age group from 20 to 29 years The reason for the somewhat higher percentage of positive liver specimens from males in Colombia than in Brazil is not known

The Possibility of Transmission from Man to Man by Sylvan Mosquitoes Bugher Boshell Manrique et al (1944) have suggested that in coffee

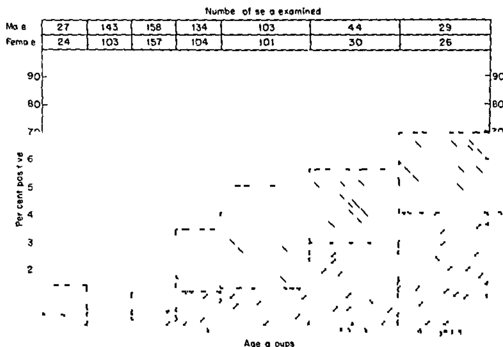


Fig 10 Results of immunity survey in a region (Ilheus Brazil) where jungle yellow fever is endemic

plantations where haemagogus are abundant transmission from human to human may take place by these mosquitoes At Chanchamayo Peru an epidemic among coffee pickers did occur in 1943 but there was no definite proof that the infection was transmitted from human to human It is also conceivable that in instances where haemagogus mosquitoes are exceedingly prevalent about houses and the epidemic is very intense this mosquito may transmit the disease from person to person within the household It is believed however that such instances are comparatively rare first because the personal histories of individuals and the analysis of data collected from immunity surveys and viscerotomy services all point to forest contact as

TABLE 28

AGE AND SEX DISTRIBUTION OF JUNGLE YELLOW FEVER IN BRAZIL DIAGNOSED BY LIVER EXAMINATION

Age groups	*Cases*	*Per cent of total*	*Male*	*Female*
0–4	14	1	10	4
5–9	30	3	24	6
10–14	34	3	30	4
0–14	78	7	64	14
15–19	72	7	54	18
20–29	282	28	233	49
30–39	242	24	202	40
40–49	169	17	146	23
50–59	95	9	86	9
60+	82	8	62	20
15+	942	93	783	159
Grand total	1 020	100	847	173

Per cent of males = 83 Per cent of females = 17

TABLE 29

AGE AND SEX DISTRIBUTION OF JUNGLE YELLOW FEVER IN COLOMBIA DIAGNOSED BY LIVER EXAMINATION

Age groups	*Cases*	*Per cent of total*	*Male*	*Female*
0–4	1	0	0	1
5–9	11	2	9	2
10–14	20	4	16	4
0–14	32	7	25	7
15–19	59	12	55	4
20–29	195	41	183	12
30–39	80	17	74	6
40+	110	23	99	11
15+	444	93	411	33
Grand total	476	100	436	40

Per cent of males = 91.6 Per cent of females = 8.4

dominating the picture and second because it seems rather unlikely that a mosquito that is essentially sylvan in its habitat would remain in or near a house during the period of extrinsic incubation of the virus or would return to the house after it becomes infectious

IN AFRICA

If the term jungle yellow fever is restricted to the disease in man contracted by forest contact and conveyed by the same sylvan mosquito that transmits the virus in the forest cycle there is no proof that this epidemiologic form of the disease exists in Africa The studies in Uganda imply the intrusion of a second vector which under the conditions prevailing in that country may be described as a semidomesticated mosquito in that it breeds principally in the axils of cultivated plants in the vicinity of houses The present concept is that this mosquito (*A simpsoni*) becomes infected from monkeys at the edge of the forest or from monkeys that maraud the banana plantations and then conveys the infection to man For these conditions to be fulfilled the intermediate vector must be present in adequate numbers and the house or village must be situated near the forest Several observations may be cited in support of this view

During the construction of a road through a part of the Semliki forest in Bwamba County Uganda from 400 to 700 workmen were employed daily The high immune rate among monkeys indicated that yellow fever virus infection was enzootic in this forest Also yellow fever was current among inhabitants of villages situated within or near these forests Yet close observation of these laborers working within the forests many of whom were not immune failed to reveal any yellow fever among them during the construction period *A simpsoni* is abundant about the villages but is rare within the forest (Hughes Jacobs and Burke 1941 Mahaffy Smithburn et al 1942) Haddow Dick et al (1950) cited two additional examples Yellow fever virus infection is enzootic in the forests about Entebbe but the human population is virtually free from yellow fever Here differing from the villages bordering the Semliki forests *A simpsoni* is exceedingly scarce The Kabale forest presents a similar but somewhat different situation As in the other forests yellow fever is enzootic and the arboreal vector *A africanus* is abundant *A simpsoni* is found in the plantations but in this instance the plantations are situated some distance from the forests and the village inhabitants escape infection The reason why man rarely becomes infected

within the forest by *A africanus* is probably that this mosquito usually bites only after sundown and it is not the custom of natives to enter or remain in the forests except during the day However it has been observed that forest mosquitoes including *A africanus* may migrate into neighboring banana plantations from the forests at night (Haddow 1945*b*) and it has been suggested convey the infection to man (Mahaffy 1949)

In contrast in South America infection with the forest virus is associated with the contact of individuals with the forest while in Uganda it is related to the propinquity of the habitations to the forest

In West Africa information is inadequate concerning the mechanism and the frequency of infection of man from virus harbored in the forests *A aegypti* is widely disseminated and it is usually impossible to eliminate transmission from man to man by this mosquito However acquired immunity in monkeys indicates that the forest cycle exists and in some localities circumstantial evidence points to the transfer of the forest virus to man (Findlay and Davey 1936*b* Durieux Boiron and Koerber 1946) As contracting yellow fever from within the forest is presumably not a frequent occurrence the age and sex incidence is of no value in speculating on the origin of the infection in contrast to the situation in South America Indeed the age and sex incidence of yellow fever in Africa presumably transmitted by domestic or semidomestic vectors appears to be somewhat different from that observed in the New World The data in this respect are rather meager but Beeuwkes (1936) in the analysis of observed cases of urban yellow fever on the West African coast found that the highest incidence occurred in the age group from 20 to 29 years Kirk (1941) likewise found in the Nuba Mountain epidemic that the attack rate was higher among males than among females and that adults were attacked with greater frequency than children It is difficult to interpret these data without more complete information on the environment and the habits of the people involved It may be concluded therefore that while human infections from the forest cycle undoubtedly occur in Uganda through the medium of *A simpsoni* and that exchanges between the forest and the human cycle probably do occur elsewhere in Africa the exact mechanism of this event is unknown

Reference has been made to the scattered and relatively low immunity rates observed in Central and East Africa extending as far south as northern Bechuanaland which suggest that the human infections may be secondary to some extraneous reservoir The clarification of this situation awaits further study

LABORATORY AND HOSPITAL INFECTIONS WITH YELLOW FEVER VIRUS

Yellow fever contracted in the laboratory can scarcely be considered as coming under the natural history of the virus or belonging to the usual epidemiology of the disease Such infections are the consequences of artificial manipulations of the virus They are of interest however in that they illustrate how under certain circumstances an infectious agent may deviate from its common route in gaining entrance to a new host These infections also tragically reveal the danger of handling a parasite that possesses the ability to enter the body through the skin and mucous membranes

Very shortly after the virus was conveyed to monkeys and investigators began to handle infectious monkey blood and tissues laboratory infections developed It is worthy of note that until a protective vaccine was discovered these infections continued to occur notwithstanding the recognized danger and the institution of all reasonable precautions

Among the 32 laboratory infections reported and reviewed by Berry and
Kitchen (1931) the source of infection was assigned as follows 20 from
contact with infectious monkey blood or tissue 4 from handling infecte
animals 3 from contact with infectious mouse brain 2 from bite o
infective mosquito and 1 unknown In only two instances when infe
tion resulted from the bite of an infective mosquito was the exact means
entrance of the virus apparent In the remaining 30 instances it may
assumed that the infection took place either through the skin by con
with infectious material or through the mucous membranes quite likel
inhalation

In addition to the 32 laboratory infections Low and Fairley (1931
ported 2 hospital infections One of these so-called hospital infec
occurred in a laboratory technician who performed biochemical tests o
blood of a person who had been infected from exposure to infected mo
The second hospital infection developed in a hospital attendant w
made a routine smear and blood count on the first patient While th
designated as hospital infections it is presumed that in both instan
infection resulted from contact with infectious blood and are therefo
lar to infections acquired in the laboratory by contact with infectio
key blood and tissues This point is of practical import as these i
do not imply that the disease may be contracted by ordinary pers

tact Indeed such an inference would be contradictory to all previo perience To quote from Reed (1902)

Without entering into details I may say that in the first place the Commission saw with some surprise what had been so often noted in the literature that patients in all stages of yellow fever could be cared for by nonimmune nurses without danger of contracting the disease The noncontagious character of yellow fever was therefore hardly to be questioned

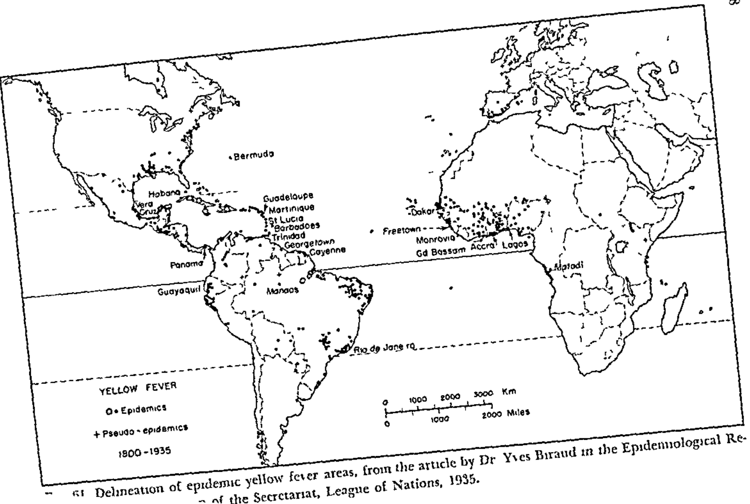
However these laboratory infections do indicate that contact with the viru is dangerous and it may be inferred that the reason the disease is noncontagious is because the virus has no natural means of escape except through the insect vector This is borne out by the experiments of Reed and his collaborators (United States 61st Congress Yellow Fever 1911) who exposed normal volunteers to the soiled linen and dejecta of yellow fever patients Although living virus may not be contained in the vomitus urine and feces or excreted through normal mucous membranes it would seem prudent to avoid contact with vomitus or sputum tinged with fresh blood Bleeding of the gums and nose is not uncommon and may occur during the infectious stage of the disease and while we are not aware of any tests having been made it is probable that the exuded blood contains active virus

Experiments on excretion of the virus by monkeys and cross infection of laboratory animals have been reviewed in Chapter 2

PAST AND PRESENT GEOGRAPHIC DISTRIBUTION OF YELLOW FEVER

It is enlightening and indeed a source of great satisfaction to compare the geographic expanse of yellow fever during the nineteenth century and first quarter of the twentieth century with the more restricted habitat of the virus today The present or recent distribution of the virus as revealed by the occurrence of recognized human cases of yellow fever and by immunity surveys in both man and lower primates is shown in Figs 58 and 59 As has been noted the virus is confined endemically to South America and Africa within the Tropics of Cancer and Capricorn and only in South America has it made occasional epizootic excursions beyond these confines The manner by which the virus is retained in its present abode has been discussed and requires no further comment

The map Fig 61 prepared by Béraud (193[illegible]) shows the location of



61 Delineation of epidemic yellow fever areas, from the article by Dr Yves Biraud in the Epidemiological Re- of the Secretariat, League of Nations, 1935.

demics and pseudoepidemics occurring between 1800 and 1935 D
this period as previously noted there were severe epidemics in the U
States and southern Europe and pseudoepidemics extended as far nort
Canada and England

THE ORIGIN OF YELLOW FEVER VIRUS

The birthplace of yellow fever virus may ever remain a matter of specul
tion but there are a number of established facts and a good deal of sugges
tive information that furnish clues to its geographic origin These are
antigenic unity of all isolated strains of yellow fever virus past and present
geographic limitations of the disease historical records of illnesses simulat
ing yellow fever geographic differences in the tolerance to infection of man
and other primates requirements for transoceanic transport of the virus
and the geographic origin of the important urban vector *A aegypti*

The fact that all strains of yellow fever virus isolated in both Africa and
South America are very closely related if not identical with one another
strongly implies a common origin There is no clinical or immunologic
evidence that yellow fever exists or ever did exist elsewhere than in South
America the adjacent Caribbean area and Africa with former seasonal
excursions into North America and Europe Consequently it seems reason
able to assume that the virus arose in tropical America or in tropical Afric
It is proposed here to summarize the information that tends to favor either
one or the other of these continents as the original home of the virus

The points favoring an American origin of the disease rest mainly upon
the recognition of yellow fever in the New World about 130 years before it
was clearly described in Africa and the lack of any records indicating that
the disease invaded Europe prior to the discovery of America Carter (1931)
though conceding that the first dependable description of yellow fever was
of the Yucatan epidemic in 1648 long before the disease was definitely rec
ognized in Africa in 1778 nevertheless concludes that yellow fever in man
previously existed in Africa but escaped attention He says On three
counts (1) the small number of Europeans among whom only could
yellow fever be recognized (2) the prevalence of severe malaria making it
difficult of recognition even among them and (3) the paucity of historical
record it seems obvious that not recording yellow fever in Africa would be
of far less negative significance than the same would be for America (Page
06)

In contrast to the lack of historical records preserved by the West African Negro tribes he points to the rather voluminous recordings of the pre Colombian American Indian civilizations as well as of the early Spanish and Portuguese invaders An extensive search through these records convinced Carter that yellow fever did not exist in the New World prior to the Conquest He attaches little importance to the apparent failure of yellow fever to invade southern Europe until sometime after the discovery of America because of the limited communications with West Africa Contrary to Carter s opinion Berenger Feraud (1890) likewise on the basis of historical record favored the American origin of the disease

However it is believed that in comparison with other evidence the early history of yellow fever may be largely discounted because of (*a*) the paucity and inconsistency of the preserved records (*b*) the difficulty of deciding from the usually inadequate description of the symptomatology and epidemiologic characteristics of epidemics whether a retrospective diagnosis of yellow fever is warranted and (*c*) particularly because of the now recognized forest cycle of the virus which may have preceded the urban epidemics of the disease in man Jungle yellow fever which occurs almost exclusively among rural populations would have escaped attention or at least recording Carter was of course unaware of the existence of jungle yellow fever and in his review of old records gave consideration only to the description of urban epidemics that fitted the pattern of *A aegypti* transmitted yellow fever In that it is reasonably certain that *A aegypti* derives from Africa and was probably introduced into the Americas during the slave trade period the American epidemic that Carter accepts as yellow fever and those that followed during the succeeding two and one half centuries were undoubtedly related to the introduction of this mosquito It is conceivable however that the virus may have been present in South American forests before the voyage of Columbus and that the disease in man came to light only after the introduction of the efficient urban vector *A aegypti* In passing judgment on this possibility it is necessary to resort to evidence other than history of the human disease

The African origin of the virus is supported by (*a*) the generally greater tolerance of the African monkeys and the native West African to yellow fever virus infection in comparison with South American monkeys and the American Indian (*b*) the requirements for the transoceanic transport of the virus and (*c*) the African origin of *A aegypti* The plant pathologists have utilized information on the relative tolerance of the plant hosts to a

virus for postulating the geographic origin of the virus for as Holmes (1 said It seems obvious that wherever a disease has existed for a long ti the most susceptible of its host plants will have been depressed in comp tive vigor Any less affected mutants from these host plants will have tend to supplant or will have supplanted the original representatives of the sp cies It seems logical that this reasoning may apply as well to mammalia viruses and their hosts In any case it is an established fact that virtually al of the African monkeys though highly susceptible to infection with yellow fever virus are relatively tolerant to the disease The symptoms produced are usually negligible and the disease is very rarely fatal except in the East African galagos On the other hand yellow fever virus commonly produces a fatal infection in a number of species of South American monkeys including the howler saimiri and aotus monkeys and several species of marmosets The evidence of a racial tolerance in man though not subject to controlled experimentation strongly suggests that the disease in the African Negro is generally of a milder character with lower fatality rates than observed in other races There appears to be no such tolerance enjoyed by the South American Indian (Carter 1931) If it be assumed that this species and racial tolerance is attributable to long experience with the virus resulting in the elimination of the highly susceptible and the preservation of the more tolerant stock it would point to a more ancient existence of the virus in Africa than in America

The mildness of the infection among other orders of South American mammals such as the marsupials is more likely due to a lack of adaptation of the virus to the host than to genetically acquired tolerance of the host to the virus

With regard to the transoceanic conveyance of the virus by sailing vessels the only former means of transport it is necessary to assume that such conveyance took place by means of or in conjunction with a mosquito vector The short incubation period and the brief period during which the virus circulates in the vertebrate host preclude the possibility of transport of the virus by man alone Moreover the mosquito responsible for conveying the irus must have been a highly domesticated species that would live and ultiply aboard ship The habits of *A aegypti* are peculiarly suited to meet ese requirements That the disease was transported by ship in conjunc on with this mosquito in later years is well established As mentioned ore there is reason to believe that *A aegypti* stems from Africa and it is bable that at some former time possibly during the early slave

this mosquito and the virus were introduced into the New World The other and more complicated alternative is that *A aegypti* was brought from Africa or southern Europe became established in tropical America encountered the virus of yellow fever as a result of which urban epidemics were initiated and the mosquito along with the virus was then conveyed back to Africa

It has been contended that the wide dissemination of the virus in tropical forests in South America indicates an ancient existence that antedates the colonization period The validity of this contention is questionable The epizootics in southern Brazil illustrate the rapidity with which the virus may spread even over areas where the forests are not contiguous Once introduced it is conceivable that the virus may have spread within a few decades over the present enzootic zone It is believed therefore that the weight of evidence suggests that yellow fever virus is of African origin and was probably transported to America during the early slave trade

The question is frequently asked Why is there no yellow fever in India and the Far East? The biologic environment for its propagation and maintenance appears to be favorable *A aegypti* is widely distributed and susceptible animal hosts both man and monkeys are abundant There is no evidence that the peoples of India possess any racial resistance to infection with yellow fever virus or unusual tolerance to the disease The rhesus monkey of India is one of the most susceptible of all the primates and is much less tolerant to the disease than any of the African monkeys Thus it would seem that the probable explanation of the absence of the disease in India or elsewhere in the tropical Far East is that the virus was never introduce Formerly there were two overseas routes that the virus might have taken reaching India from Africa—the more direct route from the east coast of Africa to India and the longer route from the west coast around the C of Good Hope Though immunity surveys have revealed the existence immunes in East African ports the proportion of immunes is low T low immunity rates imply that the disease is infrequent and rarely if reaches epidemic proportions Under such conditions the chances th disease might be conveyed by the most direct route from East Afric not very great

It has been pointed out that rather special conditions are requir overseas transport and the longer the voyage the less likely that the ditions will be fulfilled The voyage from tropical West Africa to I long and in rounding the cape climatic conditions are frequently

tered that are unfavorable to the survival of the mosquito vector For w
of a better explanation these considerations are tentatively offered

SUMMARY AND CONCLUSIONS

MAN—MOSQUITO CYCLE

In the New World the only vector involved in this cycle is a highly do-mesticated race of *A aegypti* Yellow fever resulting from this cycle formerly occurred both epidemically and endemically It was seasonally epidemic in temperate zones where the mosquito is unable to survive throughout the year and was epidemic in urban communities within the tropics having populations insufficient to support endemicity It was endemic within the tropics where the mosquito may survive through all seasons and where the populations involved were sufficiently large Endemicity was therefore limited to cities and to rural communities where conditions were peculiarly favorable for the continuous dissemination and propagation of the virus This cycle was responsible for the former prevalence of urban yellow fever in the Caribbean region and South America and for the epidemics that invaded the United States It also accounted for the epidemics in Europe during the eighteenth and nineteenth centuries The infection was transported over great distances overseas along interior waterways and other routes of commerce through the medium of infected persons or infected mosquitoes or a combination of the two After being introduced the course of the infection is conditioned by the prevalence of the vector and the relative number of susceptible humans

The chain of aegypti transmitted yellow fever in the New World was broken with the elimination of the last endemic focus in northeastern Brazil in 1934 Small inland outbreaks continued to occur in South America as a result of the introduction of the virus from the forest the last was reported in 1942

In Africa the vector varies according to region Along the west coast *A aegypti* is a proved vector and its wide distribution combined with its domestic habits and feeding preferences indicate that it is the most im-portant if not the only urban vector although several other species of mosquitoes capable of transmitting the virus are present in or about urban communities The disease is endemic but is also manifest by epidemic out-breaks Information is insufficient to delineate the zone of enden

ately but it may be surmised that endemicity is dependent mainly upon nate and other factors that favor the survival and year round existence the vector in adequate numbers

In the Anglo Egyptian Sudan other vectors *A vittatus*, *A metallicus*, *taylori* and possibly *A furcifer*, besides *A aegypti* are suspected and it is possible that some of these species may play a role in transmitting the disease in other parts of East and Central Africa

The intensive studies made in forested regions of Uganda have revealed another vector of the virus from man to man namely *A simpsoni* In these regions this mosquito is found in abundance about houses and in the edge of the forests It appears to play a dual role first of conveying the infection from monkeys in the forest to man and then after the virus is introduced into a village of transmitting it among the inhabitants To what extent this pattern of the man–mosquito cycle applies to other parts of East and Central Africa is not known

With the exception of some towns and villages in the Anglo Egyptian Sudan immunity rates revealed by surveys in East and Central Africa are considerably lower than along the west coast of Equatorial Africa It may be questioned if the relatively low and scattered immunity rates found southward from Uganda to northern Bechuanaland are compatible with endemic man–mosquito cycle yellow fever

FOREST OR ANIMAL–MOSQUITO CYCLE

Judging from human infections contracted by forest contact and immunity in primates in South and Central America this cycle has extended as far north as western Panama and as far south as Misiones Territory in Argentina It is believed to be regionally enzootic in the Amazon Orinoco hydrographic system in a forested strip along the coast of Brazil centerin about Ilhéus in forests adjacent to the Amazon Orinoco watersheds in th Guianas and in Venezuela in the Magdalena basin and in eastern Panam From the fringe of the enzootic zone periodic epizootics extend for vary distances up to 1 500 km (Fig 53) These epizootics are characterized their seasonality and rapid spread Seemingly the virus persists in one not longer than one or two seasons then moves on to completely disap within the epizootic zone until the next epizootic wave Within the enz zone there is a continuous process of wandering epizootics by virtu which a state of regional endemicity is created The only known cyc

volves primates and possibly some of the marsupials and sylvan mosqu the most important being the genus *Haemagogus* The spread of the viru the rapidly moving epizootics is attributed to the wind drift of infected m quitoes and the season to season maintenance of the virus to its prolon though not permanent retention in this vector reservoir

In Africa the forest cycle is enzootic in Uganda and it may be assum that this condition prevails throughout Equatorial Africa wherever simil forestral environment is found (Fig 61) On the west coast immune mon keys have been reported as far north as Senegal and as far south as th Belgian Congo One immune specimen was found in the Anglo Egyptian Sudan and the immunity rate in Uganda is exceedingly high No data are available for southeastern Africa It is not improbable however that as in South America epizootics extend both northward and southward from the enzootic zone Also as in South America the enzootic status appears to be created by a continuous process of wandering epizootics

The mechanism of the cycle seems to be strikingly similar in South America and Africa In both continents primates constitute the most important vertebrate host and sylvan mosquitoes constitute the vector *A africanus* is a proved and seemingly the most important vector in Africa and like haemagogus is found mainly near the forest canopy consequently it appears that most of the transmission occurs in the higher vertical strata of the forests

Jungle or sylvan yellow fever according to the definition followed in this chapter results when man is bitten by an infected forest mosquito In the Americas this is virtually the only epidemiologic form of the disease now existing It has accounted for 97 9 per cent of the human infections diagnosed by liver examination during the last 15 years It is acquired almost exclusively by direct forest contact It is suspected that a few infections may be acquired about or even within houses situated in close proximity to forests but the great majority are contracted by people entering or working along the edge of the forest It is for this reason that the incidence of the disease is much higher in adult males than in women and children This is in marked contrast to aegypti transmitted yellow fever which attacks women and children as often as if not more frequently than adult males Since jungle yellow fever represents a tangential and fortuitous transfer of the virus from the forest it follows in the wake of epizootics of the forest cycle and its mass behavior is therefore associated with these epizootics

According to the restricted definition here employed it is probable that very little jungle yellow fever occurs in Africa at least not in Uganda where the forest cycle and the human disease have been most thoroughly investigated The mosquito *A africanus*, involved in the forest cycle rarely bites man not only because it prefers monkey blood but particularly because it is a crepuscular and night feeder and human beings are not given to remain

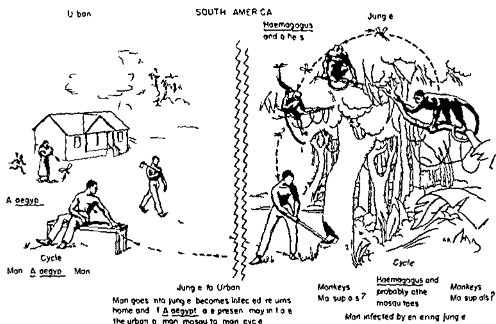


Fig 62 The drawing shows the manner of acquiring jungle yellow fever and the transfer of the virus from the forest to the man–mosquito cycle The transmission by *A aegypti* is represented as occurring outside the house whereas in all probability most of it takes place within doors The broken lines indicate that the house may be some distance from the forest

ing in the forests after nightfall The infection of man with the forest virus takes place through the intermediary of a semidomesticated mosquito *A simpsoni* which becomes infected from monkeys along the forest edge or from monkeys entering banana plantings about houses This may appear to be a rather minor distinction as man does become infected from mosquitoes that have acquired the virus from monkeys But it does exert a significant influence upon the epidemiologic manifestations It is therefore not by personal contact with the forests that man is exposed to infection but rather by the nearness of his habitation to the forest and the presence of

a secondary vector Consequently the disease is not mainly limited to adult male as it is in the neighborhood of the house that infection t place and all members of the household are more or less equally expo

THE INTERCHANGE OF THE VIRUS BETWEEN FOREST CYCLE AND MAN—MOSQUITO CYCLE

The introduction of the virus from forests into urban communities wher *A aegypti* is present resulting in the initiation of an urban epidemic ha

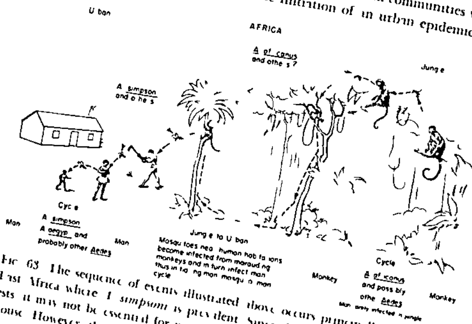


Fig 63 The sequence of events illustrated above occurs principally in parts of East Africa where *A simpsoni* is prevalent Since this mosquito may enter forests it may not be essential for monkeys to come within close proximity to the house However the infection rate in humans is related to the proximity of the house to the forest

been known to occur on several occasions in South America and probably accounted for many of the puzzling outbreaks in the past where no relation to other urban foci could be traced In such instances it is believed that the virus is usually introduced by a person becoming infected through forest contact and then coming to town while in the incubation period or early stage of the disease (Fig 62) Immediate proximity of the town or village to the forest is not an essential requirement

In Uganda and other parts of Africa where similar conditions exist village epidemics are initiated by transfer of the virus from monkeys to man by *A simpsoni* and the same vector may then proceed to convey the infection from man to man This demands that the village be situated near a forest where the virus exists (Fig 63)

Nothing is known of what actually occurs in West Africa but since both the forest and the man—mosquito cycle exist side by side it is not unlikely that some interchange of virus from one cycle to the other takes place However in the densely populated regions along the coast where aegypti are abundant there is reason to believe the virus may be maintained in the man—mosquito cycle without the necessity of reseeding from the forest

Information is yet too meager to warrant a definite opinion upon the epidemiology of yellow fever revealed by human immunity surveys in Southeast Africa

In the foregoing pages an attempt has been made to set forth the present concepts of the epidemiology of yellow fever and the natural history of the virus But a word of caution against complacency is not out of place It is well to remember that 20 years ago eminent epidemiologists believed that all the essential facts concerning the epidemiology of yellow fever were understood that the virus was limited to one vertebrate host man and that it was transmitted by a single species of mosquito *A aegypti* The opinions of 20 years ago had to be revised Those of today may also require revision There are obvious lacunae in our knowledge that need to be filled There may be wider gaps of which we are unaware

9 CONTROLLING YELLOW FEVER

by HUGH H. SMITH, M.D.
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International Health Division
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DURING THE eighteenth and nineteenth centuries yellow fever beca
the most dreaded disease in the Americas The period was marked by lar
scale migrations from Europe to the New World and by overseas milit
campaigns of unprecedented scope New towns and cities sprang up alon
the coasts from Nova Scotia to Argentina Naval and commercial shippi
expanded greatly The sailing ships because they had to carry large supplie
of fresh water offered excellent conditions for the breeding of mosquitoe
and even for the transportation of adult *Aedes aegypti* from one port to
another While the growing importance of the ports along the North Ameri
can seaboard facilitated trade with the West Indies Central and South
America it also created ideal circumstances for the implantation of yellow
fever

From early experience with yellow fever it seemed that the disease was
confined chiefly to urban communities along the coasts or on navigable
rivers In the tropics communities that received a steady influx of nonim
mune persons either newborn infants or newcomers from regions free of
yellow fever were likely to harbor the disease on an endemic basis Havana
was a classic example of this and for long periods yellow fever was endemic
in Guayaquil Veracruz and Sao Salvador do Bahia as well The inhabitants
of these cities became immune to or died of yellow fever at an early age
Consequently among adults it was the foreigner or newcomer who was in
jeopardy From time to time when military operations involving large num
bers of susceptible troops were undertaken in regions where yellow fever
was endemic intense outbreaks occurred Admiral Vernon and General
Whitworth in 1741 are said to have lost 8 431 of 12 000 men at Cartagena
The disease was very severe in the West Indies in 1793 and the following
years Among the European troops it is said nearly 700 officers and 30 000 soldiers died of it (Scott 1939)
The classic epidemics of yellow fever usually followed upon the introduc
tion of the infection by ship to cities of the temperate zone whose popula
tions were largely nonimmune Such epidemics produced widespread dread
and panic The general fear of yellow fever reflected in old accounts is im
pressive Dr E D Fenner opens his account of the New Orleans epidemic
of 1853 "Once more it becomes my painful duty to recount the rise prog

less and mournful ravages of the terrible pestilence, which since the beginning of the present century, has so often and so severely scourged our devoted city" (Fenner, 1851)

New York, Philadelphia, Baltimore, Norfolk, Charleston, and many other cities were repeatedly attacked Having no knowledge whatever as to the etiology of the pestilence, the individual's only effective resort was flight to uninfected areas When undertaken too late, this action served only to disseminate the disease to other communities The quarantines imposed in early days were generally useless In the words of Dr. Louis G. LeBeuf "No measure ever controlled the situation; no amount of disinfection ever helped, and when the entire truth was told, the only relief which came was when the frost of November appeared as a Heaven sent Nemesis to our dreadful scourge' (Augustin, 1909)

With the conversion from sail to steam in ocean travel the number of outbreaks of yellow fever in the United States markedly decreased Those that did occur were for the most part limited to the states along the coast of the Gulf of Mexico and to the towns along the principal southern rivers Nevertheless the disease remained a source of dread because not only were means of effective control totally lacking but the nature of the disease was unknown Dr F D Fenner in 1853 summed up the unsatisfactory state of knowledge about yellow fever when he referred to

> the great questions which have ever agitated the medical profession and the community at large concerning the true cause and real nature of yellow fever—whether it be a disease sui generis, or only one of the protean forms or varieties of endemic fever indigenous to southern climes—whether it be of foreign or domestic origin in the United States—whether it be contagious or noncontagious—transportable or not in persons or goods from places in this country, where it prevails epidemically, and communicable to persons who reside at distant and altogether different places where this disease was never known to prevail (Fenner, 1854)

The argument as to whether yellow fever is contagious continued year after year The nature of this dispute was well stated by Dr Bennett Dowler in 1878.

> At the beginning of the present century and for some years after, the yellow fever element was so mingled with the great concerns of humanity, that it excited the public mind to an unexampled degree, in the cabinet and in the field, in the legislative halls and in the medical schools, both at home and

abroad and in the colonial governments It had long been the conqueror of armies and navies and at one time it threatened to desolate the peninsula of Europe Its contagiousness was a leading topic, on which reports pamphlets and books went forth raging like the epidemic itself Neutrality was scarcely possible in a matter so deeply involving the interests passions and transactions of humanity Opinions founded on mere hypothesis concerning the cause of this malady which remains to this day unknown were not for that reason less but even more positive and dogmatic Affidavits and affronts certificates and satires logic and duels personal contagion and personal invective bad air and worse legislation divided the professional and non professional public on this subject The non contagionists however greatly outnumbered their opponents They for the most part controlled the legislation of the States of the Union by their efforts or their arguments But no sooner were they off their guard than the contagionists appealed to the fears of the people and urged the legislature to do something for the protection of the people by making laws against the importation of yellow fever whereupon new laws were often enacted with no effect in this behalf (Keating 1879)

By the turn of the century the bacteriologic concepts of Pasteur and Koch altered the thinking of health officials charged with protecting communities from yellow fever Progress in the control of other epidemic diseases made it appear likely that yellow fever too could be brought under domination Broader understanding of communicable diseases gave a clearer insight into the mechanism of the phenomena of epidemics Old observations on yellow fever were carefully restudied New ones were made At this time Dr Henry R Carter wrote It is assumed that the infection of yellow fever may be conveyed directly from one sick of that disease to his environment it being so generally admitted that it seems a waste of time to argue it (Carter 1900)

The concept of yellow fever as a contagious disease led during the last decades of the nineteenth century to the institution of control measures thought to be effective in the management of other communicable diseases These measures consisted principally in quarantine in the isolation of the patient from the community and in the disinfection of houses and objects with which the patient had contact

For disinfecting houses the use of sulfur dioxide gas was recommended Steam sterilization and boiling or soaking in germicidal solutions were considered effective in the case of bedding and clothing Since rotting wood was

believed to be an especially bad medium of infection outside prem
cleared of trash and leaves The ground was then thoroughly wet
bichloride solution, or covered with chloride of lime Outside buildi
preferably disinfected by fire (Carter, 1899)

Unfortunately under endemic conditions, or when epidemic yello
had become widespread in an urban community, these measures turn
to be totally ineffective

EARLY ANTIMOSQUITO CAMPAIGNS

CUBA

For two centuries coastal cities of the United States lay open to attack yellow fever, imported as a rule from Havana During the latter half of th nineteenth century a cast iron commercial quarantine operated against th West Indies in all Gulf ports, each summer, and every few years, when yellow fever, in spite of precautions, would get into the United States, the same inflexible quarantines went into effect among the various cities and states along the Gulf coast The belief grew that if, in any way, Havana could be rid of yellow fever, the disease would cease to be a menace to the southern states (Gorgas, 1905) With this in mind, the American military government during its stay in Cuba concentrated its effort on the control of yellow fever and paid great attention to the improvement of hygienic conditions all over the island Since it was believed that Havana was the only endemic seedbed in the island, the energies of the military government were focused on this city

By improving the sanitary conditions it was hoped that yellow fever could be greatly diminished and possibly, in the course of a number of years gradually eliminated In Havana, street cleaning garbage disposal, and yard cleaning were vigorously undertaken A few months after the inception of this program in 1899, garbage collection became a regular practice and the streets were as clean as those of any modern city The internal sanitation of houses and the organization of the sanitary department for the reporting and control of contagious and infectious diseases took more time

In the early part of 1899 the first year of military occupation very little ellow fever occurred The war had halted immigration to Havana, and e nonimmune population was reduced to a low figure Only seven deaths om yellow fever were reported in the first 7 months of the year

August however Spanish immigrants began to pour into the city 12 000 people arrived before the end of the year At once yellow feve up By December a severe winter epidemic was under way This ep continued through 1900 Some 1 400 cases were reported The imp sanitary conditions had lowered the general death rates but did not the incidence of yellow fever (United States 61st Congress Yellow Fe 1911)

In the summer of 1900 a commission of United States Army medical cers headed by Major Walter Reed was sent to Cuba to investigate the low fever problem The commission after several months of elaborate a careful experimentation provided clear proof of the theory advanced Dr Carlos Finlay of Havana in the year 1881 that yellow fever is tra mitted by the stegomyia mosquito (then called *Culex fasciatus* Fabricius an later *Aedes aegypti*) In February 1901 Major Reed read a paper before th International Sanitary Congress in Havana giving the results of his experi ments (United States 61st Congress Yellow Fever 1911) Since the idea of mosquito transmission was contrary to the prevailing views on the subject the paper received scant credence Even Major W C Gorgas MC United States Army then Chief Sanitary Officer of Havana who had followed the progress of the commission s investigations and was convinced that the mos quito could convey yellow fever was hardly prepared to believe that it was the only way or even the ordinary way in which the disease was transmitted (Gorgas 1905)

All ordinary sanitary measures for the preceding two years however had failed to control the disease Yellow fever at the beginning of 1901 was as bad as it had ever been in Havana at that time of year Every section of the city was infected

Although he had little hope of accomplishing much it seemed to Gorgas hat the new knowledge of mosquito transmission should be put to a test le discussed with Walter Reed the practical application of the new discov) It did not seem likely to either of them that the adult mosquito could destroyed in sufficient numbers to be effective in the control of yellow er Information on the life history of the stegomyia mosquito was meager The importance of the discovery that yellow fever is transmitted by the of a certain species of mosquito did not fail to attract the prompt atten of General Leonard Wood Military Governor of the Island of Cub elf a physician and formerly a distinguished member of ient of the United States Army In I

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was at once subjected to a practical test in the city of Havana The necessary funds were authorized and work began on February 1 1901

The Reed commission had demonstrated that in order to become infected the mosquito had always to bite some yellow fever patient within the first 3 days of illness It appeared evident that if the mosquito could be prevented from reaching every case of yellow fever in Havana the disease would disappear To accomplish this new regulations were set up requiring that all cases of yellow fever in the city be reported to the central office of the health department Such cases were seen without delay by an official commission of diagnosticians If their verdict was yellow fever the patients were at once removed in a carefully screened ambulance to screened wards of Las Animas Hospital As a choice the patient could stay in his own home in a room which was screened by carpenters sent by the central sanitary office

This method could not be carried out completely as only the exceptional patient could be reached during the initial chill Generally the patient had been sick 1 or 2 days before he came under observation A number of mosquitoes might have bitten him before he could be isolated behind screens As it would be 12 to 14 days before these mosquitoes would become capable of transmitting infection there was ample time to attend to them As soon as the patient was removed the house in which he had fallen sick and all the contiguous houses were fumigated with pyrethrum or sulfur fumes to kill the mosquitoes To ensure the effectiveness of the fumigation the cracks and crevices of the house were stopped with paper and paste Sulfur although a most effective mosquito killer was difficult to ignite Furthermore the fumes of sulfur tarnished gilt metals and injured light colored fabrics Pyrethrum did not have these disadvantages but was not so lethal to the mosquito Generally it simply intoxicated but did not kill the mosquito For this reason buildings were opened within 2 hours after the burning of the pyrethrum and all mosquitoes carefully gathered up and burned

In Havana there were many cigar factories and tobacco storehouses that had to be fumigated As sulfur and pyrethrum affected the flavor of the tobacco neither could be used After much testing it was found that the fumes from burning tobacco stems did not hurt the tobacco and were deadly to the mosquito The stems were a waste product in cigar manufacturing and could be obtained in large quantities at little cost

It was known that all species of mosquitoes had to spend 8 to 9 days in a larval stage and that while in the larval stage they lived in water Therefore bodies or collections of water were necessary for the development of the

mosquito The stegomyia mosquito was observed to prefer clear, clean wa
such as is supplied by rain water caught for domestic purposes The pip
water supply in Havana served only a small portion of the city area By f
the larger part of the population obtained its water supply from rain wat
stored in cisterns, tanks, and various other receptacles It was decided to sto
mosquito breeding in all such places

The city was divided into 20 inspection districts with a sanitary inspector
in charge of each The inspector was required to visit each house in his dis
trict once a month, make a careful inspection with regard to mosquito breed
ing, and report his findings to the central office on a printed form An order
was issued making it a sanitary nuisance for any householder to have mos
quito larvae on his premises The health officer was given authority to im
pose a fine for such a nuisance, the fine was collected by the Cuban courts
and the proceeds deposited in the Cuban treasury The health officer had
authority to remit the fine at any stage of the proceedings In practice, the
threat of a fine to the householder was generally sufficient to bring about
prompt abatement of the nuisance

As cisterns and household receptacles for storing water were necessary to
a large portion of the population it was provided that all water containers
be covered in a manner such as to prevent the mosquitoes from gaining ac
ess to them The tops were covered except for a small hole protected by
reen wire through which water could enter Spigots were installed so that
ater could be drawn off at the bottom This was done at public expense
quads of workmen with materials loaded in wagons were on call at the
ntral office ready to go out when summoned by sanitary inspectors
It was the inspector s duty to empty all vessels containing larvae and
plain the facts about mosquito breeding to the householder Each in
tor had a squad of five men and one of these men carried a sufficient
unt of oil to pour upon any pools or puddles about the premises Old
les and cans were removed from yards The inaccessibility of roof gut
found to be common breeding places for stegomyia mosquitoes added
ly to the difficulties of the inspectors
e results of the antimosquito campaign were surprising and impressive
e 10 years preceding the occupation of Havana there had been an aver
more than 500 deaths per year from yellow fever in the city and 2
fter the United States Army had taken possession of the
ll 310 deaths per year from yellow fever
uary 1901, yellow fever

seven deaths from the disease in February five in March one none in April May or June one in July two in August and in September the last case of yellow fever occurred This first conquest of yellow fever in an endemic center convinced the sanitary authorities that the mosquito not only could convey yellow fever but that it was the ordinary way and the only way at least in Havana for conditions in that city during 1901 were as favorable for a yellow fever epidemic as they had ever been (Gorgas 1915)

From the experiments of the Reed commission and from the results of the Havana campaign it was evident that the causative agent of yellow fever had to be introduced into a locality either in the body of an infected individual or in the body of a female mosquito that had become infected by the agent It was concluded that if these two sources of infection could be kept out of a community yellow fever could never occur there With this object in view a quarantine was established in Havana Any ship coming in with yellow fever on board was placed in quarantine The vessel itself was fumigated so as to kill all the mosquitoes All the nonimmune crew were kept in a quarantine station for a period of 6 days If none of the men became ill during that time all were released

Havana continued free of yellow fever during 1902 1903 and 1904 In 1905 another outbreak got started but was promptly checked by application of the methods of Gorgas In 1909 the whole Island of Cuba was declared free of yellow fever and has remained so ever since

BRAZIL

During the last decades of the nineteenth century the cities of Brazil expanded rapidly attracting large numbers of European immigrants fully susceptible to yellow fever The conditions in most of the Brazilian cities were favorable to the maintenance of yellow fever on an endemic basis Rio de Janeiro which had grown very rapidly and which had a population of almost 800 000 at the turn of the century suffered cruelly from the disease The number of deaths reported from yellow fever usually amounted to several hundred each year and reached several thousand in severely epidemic years

When news of the Havana experience reached the government of Brazil the authorities made plans to free the city of Rio de Janeiro from its evil reputation as a yellow fever center In March 1903 Dr Oswaldo Cruz a bril

liant young Brazilian medical man was appointed director general of national health department Under his leadership the health services we reorganized, funds for an anti aegypti campaign granted, and control wo promptly begun The methods that had proved effective in Havana quick



Fig 61 Inspection of the harbor at Rio de Janeiro by the Brazilian Yellow Fever Service in 1931

brought favorable results Yellow fever deaths in Rio de Janeiro during campaign were as follows

Year	*Deaths*
1903	534
1904	48
1905	298
1906	42
1907	39
1908	4
1909	0

st cases of yellow fever contracted in the city of Rio de Janeiro itself d in 1908 After that, for a few years occasional cases were introd from infected ports to the north (Fig 61)
10 the state government of R

a commission to control yellow fever in the capital city of Belém A successful campaign was conducted for that purpose from November 1910 to October 1911 In 1912 the States of Amazonas, Pernambuco, and Bahia organized similar control services for their principal cities (Couto and Rezende, 1912)

MEXICO

In Mexico, Dr Eduardo Liceaga, President of the Superior Board of Health of the republic, was one of the first to grasp the importance of the mosquito transmission of yellow fever He organized control campaigns based upon the mosquito doctrine in all of the towns in which yellow fever appeared to be endemic, with especial attention to the larger seaports The measures employed involved the isolation of patients, the thorough disinfection of dwellings by sulfur dioxide, the covering of drinking water reservoirs, and the use of petroleum as a larvicide Veracruz was considered to be the oldest and most permanent focus of yellow fever in Mexico The antimosquito organization in that city proved so effective that James Carroll (1907) was able to say

> In Mexico yellow fever has been eradicated from its endemic focus at Vera Cruz through the able efforts of Eduardo Liceaga, the President of the Superior Board of Health, whose complete grasp of the problem and whose enlightened and energetic action has added support to the mosquito doctrine, and would have controlled the disease absolutely if the same means of enforcement were available in Mexico as in Cuba in 1901

Although yellow fever was not permanently eradicated from Veracruz it was for a time brought under control, again clearly demonstrating the efficacy of anti aegypti campaigns

Merida in Yucatán offered a more difficult problem due to the existence in that city of over 30,000 water tanks With the methods then employed, mosquito breeding in these tanks was not so easily controlled as in Veracruz

The campaign in Mexico was also extended to the Pacific coast ports with complete success (Liceaga, 1910)

PANAMA

With Gorgas s experience in the sanitation of Havana in mind, Surgeon General Sternberg recommended that Gorgas be placed in charge of a simi

lar campaign in the Isthmus of Panama to prepare for the building of th canal by the United States government At the request of the Isthmian Can Commission in 1901 Gorgas went to Panama to get an idea of the sanitar conditions and to formulate a general plan of action Gorgas who did not view the situation lightly commented

In my opinion the sanitary problems are grave The question of eliminating yellow fever from an endemic focus has only been once before successfully managed and that was at Havana And from this successful work to argue that it is easy and simple I think is not warranted Personally I believe it can be done and I approach the work with great hope of success but I know that it will be neither easy nor simple that we will meet with many disappointments and have to modify our plans many times (Gorgas 1901)

From the best statistics Gorgas could obtain the French during their at tempt to build the Panama Canal lost yearly by death from yellow fever about one third of their white employees (Gorgas 1915) During the first ear of Gorgas s campaign he therefore directed most attention to the con ol of that disease The two principal foci of infection were the towns at ther end of the isthmian railway Colon and Panama Since in Havana orgas had become convinced of the value of fumigation when he com nced work in the isthmus he relied principally on that method In the ital city then a small town the plan was to fumigate every house within mited time and thereby at one stroke get rid of all infected mosquitoes month was required to complete this widespread fumigation but cases ellow fever continued to occur The procedure was repeated a second Finally it was decided to concentrate on a campaign against the breed ces of stegomyia

1904 there were some 40 000 people in the territory that came under nitary control of the Canal Commission about 6 000 in Colon about in the city of Panama and some 10 000 scattered in 22 villages along road The health conditions were at about the same level as in neigh tropical countries A few cases of yellow fever occurred now and th sharp outbreaks at intervals Malaria was common Colon had supply or sewerage system and was built in a swamp Every house al water barrels for collection and storage of rain water In accord the terms of the treaty the Republic of Panama agreed that of the cities of Panama and Colon sho ary officer of the Isthmi

Gorgas introduced systems as far as these could be applied locally on t plan evolved in Havana (Gorgas 1907 Le Prince and Orenstein 191 Health departments were established in each town Mosquito inspecti districts were set up All domestic water containers were made mosqui proof All other collections of water were either eliminated or oiled unacclimatized persons sick with fever were isolated behind screen wi In order to prevent the introduction of yellow fever from the outside strict quarantine was established at the two ends of the zone Colon a Panama

The sanitary department as a bureau of the local government with access to the chairman of the commission in Washington except throu *local authorities was greatly handicapped It began work in 1901 with* adequate supplies and money Canal Zone officials with no sanitary traini looked upon the ideas of the sanitary officers with regard to mosquito wo as wild and visionary They could not be expected to take a favorable vie of the antimosquito campaigns With a flare up of yellow fever in June 190 these officials recommended to the Secretary of War that Gorgas and h chief associates be replaced by men with more practical views Fortunate President Roosevelt who was familiar with the Havana campaign refus to remove Gorgas When the sanitary department was given higher status an independent bureau reporting directly to the chairman of the comm sion the chief administrative difficulties disappeared

A law was passed to punish the harboring of mosquito larvae in priva premises Colon and Panama were divided into districts each supervised an inspector With the techniques used in Havana rapid results were o tained During the fall of 1905 yellow fever rapidly decreased and by N vember the last case of this disease had occurred in the city of Panam One more case of yellow fever occurred in Colon during May 1906 but th until many years later proved to be the last known infection originating the isthmus

The lesson demonstrated in Panama was that domiciliary sanitary me ures involved in finding the sick in isolating them and in fumigating th houses to destroy the mosquitoes even if it be successful is many fold mo difficult costly and troublesome than the work against the breeding plac of the stegomyia and also in a town in which the disease is endemic it e cites infinitely more opposition from the population (Carter 1909)

UNITED STATES

The last known outbreak of yellow fever in the United States occurred in New Orleans in 1905 In July of that year it became apparent that a serious epidemic was in the making Neighboring states and cities immediately imposed strict quarantines The local medical society had plans to cooperate with the city health department in an all-out campaign against stegomyia mosquitoes Immediate steps were taken to provide a fever isolation hospital An appeal was made to the medical profession for early notification of all fever cases to the board of health Members of the medical society were urged to arrange for all patients to be screened from mosquitoes and or their premises to be fumigated

Early in August at a meeting of the medical society it was determined at in spite of local efforts the fever was still making headway Strong recom endations were made to the city board of health that the United States blic Health and Marine Hospital Service be called in to take complete arge of the control campaign As a result Dr Joseph H White was as ned by the service to direct the city s antimosquito measures Within a weeks the intensity of the epidemic declined The infection was stamped in November It was estimated that a total of 3 402 cases with 452 deaths occurred during the epidemic The deaths were distributed as follows 57 August 220 September 111 October 58 November 6

ce then the cities of the United States have been spared the ravages of v fever This freedom from yellow fever has not come however from by the cities to protect themselves but from the removal of the ic centers in the Caribbean area

owing the successful sanitary campaign in Panama the outlook was it in 1909 that Gorgas felt justified in saying

strides in diminishing the spread of yellow fever all over the world een made in the last ten years Ten years ago the four principal cen yellow fever in the world were Vera Cruz Havana Rio de Janeiro and quil Of these Havana and Rio de Janeiro were much more important of infection than Vera Cruz and Guayaquil From Havana yellow s practically been eliminated and within the last six months Rio de has been almost as successful In Vera Cruz yellow fever has become instead of being endemic Sporadic cases within th in several different parts of tro

coast of Africa, but nothing comparable with what it was ten years ago It seems to me that yellow fever will entirely disappear within this generation and that the next generation will look on yellow fever as an extinct disease having only a historic interest They will look on the yellow fever parasites as we do on the three toed horse—as an animal that existed in the past, without any possibility of reappearing on the earth at any future time (Gorgas 1909)

TESTING THE KEY CENTER THEORY

Sanitary officials charged with responsibility for public health in Far Eastern countries were quite disturbed over the menace of yellow fever that they would face upon the rerouting of shipping after the opening of the Panama Canal Mr Wickliffe Rose, director of the newly organized International Health Commission of The Rockefeller Foundation, on a trip to the East early in 1914, was impressed by the anxiety about yellow fever Upon his return to New York, Mr Rose invited General Gorgas, Dr Henry Rose Carter, and Dr Joseph H White, well known sanitarians with great experience in yellow fever control, to discuss with him the feasibility of eradicating yellow fever from the world

So far as the United States was concerned the threat of yellow fever had to a large extent been removed by the eradication of the disease from Ha vana The countries south of the United States were however, still in a tate of constant apprehension The coast of Brazil, the Amazon Valley, the Caribbean region, and the west coast of South America from Peru to Mazat án, Mexico, were subject to invasion at all seasons Throughout this region e danger of yellow fever required constant vigilance on the part of quar tine officers and stood as a serious handicap to commerce and general eco mic development

Gorgas, Carter, and White held the view that a few endemic foci were seedbeds of infection and that if the disease were eradicated from these ould disappear from all other points As of 1914, the known foci of ow fever were Guayaquil, Ecuador, Mérida, Yucatán Manaus Para ambuco, and Bahia, Brazil possibly La Guaira, Venezuela, and some ports in the southern Caribbean, and a West African focus or groups i on the coast from Sierra Leone south and east along the Gulf of a

as thought that an urban population of about 50,000 was the usual

a year or two but in 1918 the work was resumed under the direction of General Gorgas In Guayaquil it seemed advisable before undertaking large scale control measures to subject yellow fever and other baffling infections giving a similar clinical picture to careful laboratory study in order to find out more about the true etiology of the disease This investigation was entrusted to a commission composed of Dr Arthur I Kendall Dean of Northwestern University Medical School chairman Dr Hideyo Noguchi of The Rockefeller Institute for Medical Research Dr Mario G Lebredo of Cuba Dr Charles A Elliott and Mr Herman F Redenbaugh also of Northwestern University Medical School This commission provided with laboratory equipment arrived in Guayaquil in July 1918 Within a brief period Dr Noguchi succeeded in isolating an organism to which he gave the name *Leptospira icteroides* which he believed was the cause of yellow fever This organism closely resembled *Leptospira icterohemorrhagiae*, the causative agent of Weil s disease (Noguchi 1919*a*)

Continuing his research Noguchi (1919*b*) soon reported that *L icteroides* could be transmitted to guinea pigs by the bite of *A aegypti* mosquitoes that had previously fed on a yellow fever patient or on another guinea pig infected by the same organism This finding of course again emphasized the importance of antimosquito measures for the control of yellow fever

Noguchi (1920) found that the use of a potent immune serum in the treatment of experimental infections of guinea pigs with *L icteroides* gave definite protection When administered during the period of incubation the serum was capable of completely preventing the development of the disease Experiments also showed that the injection of killed cultures of *L icteroides* into susceptible animals conferred on them a state of immunity which endured for at least 5 or 6 months (Noguchi and Pareja 1921) Both the serum and the vaccine were put to trial in humans without delay Noguchi (1925) summarized the results obtained in 237 yellow fever cases treated with his serum and gave an account of the use of the vaccine in Ecuador Mexico Central America and Brazil in which places some thousands of people received inoculations As a result of this experience Noguchi claimed that the efficacy of the anti icteroides immune serum and of the vaccine had been substantiated

In November 1918 Dr M F Connor who had been selected to direct the control campaign in Ecuador arrived in Guayaquil After a brief period of planning in cooperation with the government health officials active operation began on November 25th The plan of work closely followed the previ

ously successful campaigns with all energy concentrated on the elimin
of *A aegypti* breeding In addition to the isolation of patients and the f
gation of premises both supervised by the national department of he
the principal procedures were the mosquitoproofing of water tanks
placing of larva eating fish in cisterns and other large water containers
the detection and destruction of mosquito larvae by means of weekly ho
inspections The results were spectacular For generations yellow fever h
been present in Guayaquil and for 8 years the average number of cases p
year had been 259 In 1918 there were 460 cases By May 1919 6 month
after control work began the last case of yellow fever had been recorded
From then up to the present Guayaquil has remained free of this disease
The suppression of mosquito breeding in the principal city was followed by
the spontaneous disappearance of the disease from the surrounding towns
and villages (Connor 1920 Sawyer 1937)

The program of the Yellow Fever Commission included intensive work for the control of yellow fever infection at any point where it may be discovered When an epidemic of yellow fever made its appearance in Guatemala in June 1918 the International Health Board (which had just superseded the International Health Commission) tendered its services to the government of Guatemala to assist in bringing the epidemic under control This offer was accepted and in July when Dr Joseph H White on leave from the United States Public Health Service undertook the task he found that the disease had spread to 17 communities on or near the west coast The infection seemed to have been introduced into Guatemala from south western Mexico Given full authority by the government Dr White took immediate action Quarantine was established and maintained suspected cases were promptly isolated the houses from which they had been removed ere fumigated and measures were inaugurated and systematically carried ut for the destruction and control of the breeding places of *A aegypti* mosquitoes By September the outbreak was under complete control and after cember no further cases occurred

During 1919 yellow fever was reported from Honduras El Salvador and xico Upon request of the Central American countries a trained field e was made available After a few months of control work the disease was ned to a small number of sporadic cases in El Salvador pon invitation of the Mexican government a coopera ontrol of yellow fever in Mexico ng the latter h

Tampico to Progresso in the east, and from Hermosillo to Tapachula in the west

In August 1921, three cases were reported in St John's College just outside Belize British Honduras Vaccine and serum were sent immediately, and Dr F I Vaughn was transferred from Mexico to assist in combating the epidemic By mid November 20 cases had occurred, but the outbreak was definitely checked

Operations in each of the Central American countries and in Mexico were carried out under joint administration Each government created a yellow fever commission under its national department of health The Rockefeller Foundation Yellow Fever Commission was given representation on each of these national commissions Under the direction of Colonel T C Lyster control measures in Central America and Mexico were administered as a unit The campaign opened in El Salvador, Nicaragua Guatemala, and Honduras in 1920, in Mexico in January 1921, and in British *Honduras in August 1921*

The Mexican government had been active on its own account during the previous year and had done much to reduce the severity of the epidemic that had swept over the eastern part of the country The problem was enormously simplified by permanently sealing the domestic water tanks and by introducing larva eating fish into all containers holding sufficient water to maintain fish life Systematic mosquito control was also undertaken on the Mexican Pacific coast

The joint campaign in Mexico was vigorously pressed during 1921 No large outbreaks were reported, but infection continued to smolder throughout a wide area Official figures of cases in all Mexico fell from 505 in 1920, and 115 in 1921, to 11 during 1922 Control operations were formally closed in November 1923 no cases of yellow fever having been reported since *December 1922*

In Central America, as no cases had been reported since 1921, active cooperation in control measures ceased in July 1922 In June 1924, however, another outbreak was reported in El Salvador The national health department at once intensified its antimosquito work, and a representative of the International Health Board was sent to study the situation On October 1, a cooperative control campaign was launched The last case was reported shortly afterward

It could not be determined whether this outbreak in El Salvador was due to reinfection from *without or whether the disease had been continu*

ously present since its apparent disappearance in 1921 After this outbreak however there was no recognized yellow fever in Mexico or Central America until the 1918 outbreak in Panama

Early in 1919 an extensive epidemic of yellow fever broke out in the Department of Piura just across the Ecuadorian border in northern Peru and in the next 12 months spread unchecked over a considerable area The epidemic involved more than 3 000 cases with from 500 to 600 deaths Under the direction of Dr Henry Rose Carter of the United States Public Health Service the government health services organized energetic measures for the control of *A aegypti* breeding throughout the infected area As a result yellow fever disappeared from the province by the end of October 1920 Dr Noguchi visited the region during the outbreak and confirmed his earlier findings made in Guayaquil and Mexico by isolating *L icteroides* from cases diagnosed as yellow fever

In February 1921 yellow fever reappeared in Peru at a point south of the region previously infected The International Health Board began cooperation with the government of Peru in control measures which were supervised by Dr Henry Hanson The epidemic spread south to the Departments of Lambayeque and Libertad which had a dense nonimmune population and an extremely high aegypti index All effort was centered on the control of *A aegypti* breeding

An attempt to teach the people to prevent mosquito breeding on their own premises failed Efforts to keep water containers covered were equally unsuccessful Straining the water (which in that country was too precious to be thrown out) involved an amount of labor that made it impracticable It was found finally that if two to four small fish of local species were placed in each 10 to 15 gallon container the problem was greatly simplified A common fish called *life* (*Pygidium punctulatum piurae* E) proved to be the most satisfactory and was the one most often used in Peru (Hanson and Dunn 1925) The 750 000 fish distributed in this drive conquered the epidemic The last case was reported in Peru in July 1921

During the early stages in the development of mosquito control measures fish played an important role in practically all the campaigns Fish were used as an auxiliary but very effective measure in the campaign against *A aegypti* in Guayaquil in 1918 and 1919 Le Prince in 1920 adopted the use of fish

wherever possible Where this could not be done, the introduction of fish gave highly satisfactory control

In Merida, Yucatán, the stone cistern constructed under the patio was the preferred breeding place of *A aegypti* To free these of mosquito breeding fish were used In May 1921, Dr M E Connor reported that in 12,324 water containers in which fish were used, inspection failed to reveal a single one harboring larvae or pupae In Nicaragua, El Salvador, and Guatemala, fish played an important part in mosquito control (The Use of Fish for Mosquito Control, 1924)

In each locality a special study was made of the kinds of fish available and of the conditions under which they could be used It was soon found that indigenous fish were preferable Small fish of the family *Poeciliidae,* widely distributed throughout the tropical and temperate zones, were the ones most extensively used Later on, with the improvement of techniques, the use of fish in *A aegypti* control was largely discontinued

In 1923 an outbreak of several hundred cases in Bucaramanga, an interior town of Colombia, was investigated by Dr Joseph H White The diagnosis of yellow fever was confirmed, although the source of the epidemic remained a mystery Control measures promptly begun, quickly brought about a suppression of the disease

In Brazil the government health services had been engaged in campaigns to control yellow fever for some years Early in 1919 epidemics had broken out in six of the northern states of the country The national government organized a central yellow fever commission, and each state where the disease was known to exist appointed a similar body The anti aegypti campaigns by the end of 1920, resulted in confining the extent of the infected area to a coastal strip between Bahia and Pernambuco

The following year an epidemic was reported in the State of Bahia Apparently unrecognized for some months it had spread over a considerable area Cases were also reported in Natal, Rio Grande do Norte, and Pôrto Calvo, Alagoas Shortly after this, an extensive outbreak occurred in the State of Ceará Fortaleza, its capital, appeared to be the focal point

In May 1923, the federal government of Brazil invited the cooperation of The Rockefeller Foundation in a systematic attempt to exterminate yellow fever in the northeastern part of the country This was the beginning of a 27 year joint program of investigation and control of yellow fever in Brazil Dr Joseph H White, who was put in charge of the work, immediately made a survey of the situation and measures were introduced to improve the con

trol of *A aegypti* in the infected area Later this work was taken over successively by Dr M E Connor and Dr F L Soper

Eleven control posts with several substations were established in the port cities and at Manaus Key to the Amazon region Favorable results were rapidly obtained and the incidence of reported yellow fever declined so satisfactorily that by 1925 all but four of the 11 stations had been closed The belief that cleaning the big port cities would automatically end the infection in the interior appeared to have been confirmed once again

In 1926 however the movement of nonimmune troops through the interior of northeast Brazil resulted in a flare up of yellow fever in several states (Soper 1938*b*) Outbreaks were discovered in the States of Bahia Minas Gerais Rio Grande do Norte and Parahiba These outbreaks were brought under control and in the last half of the year 1927 all areas were so far as known free of yellow fever As no other focus of yellow fever was then recognized on the South American continent the possibility of final eradication of the infection appeared bright indeed

The optimism of late 1927 and early 1928 was dispelled by the discovery of new cases in March and April in the States of Sergipe and Pernambuco In May came the entirely unexpected report of yellow fever in the capital city of Rio de Janeiro which had been free of the disease during the 20 years following the memorable campaign of Oswaldo Cruz Rio de Janeiro was almost 1 000 miles from the nearest known infected area in northeastern Brazil and no satisfactory explanation could be made at the time as to how the virus had been introduced

Knowing that the existence of yellow fever in the capital would have serious consequences because of the large number of nonimmune persons residing there the national department of public health of Brazil quickly mobilized its resources and a strenuous campaign was organized along the lines so successfully employed by Oswaldo Cruz (Fraga et al 1930)

The importance of large cities as sources of infection for the surrounding towns and ports is well demonstrated by the events of 1929 when during the peak of the Rio epidemic yellow fever was diagnosed on board ships at various points along the South American coast from Buenos Aires to Belem Pará and even at Manaus on the Amazon River covering a distance of almost 4 000 miles (Soper 1938*b*) Yellow fever was recorded from 42 places in the State of Rio de Janeiro alone

With the reorganization of the control service the epidemic in the city

738 cases and 136 deaths (Fraga et al 1930) The last secondary extensions of infection attributed to the Rio outbreak were stamped out by May 1931

During 1928 and 1929 when cases of yellow fever again appeared in the northern cities of Bahia Recife and Belém health authorities were forced to realize that although the sanitation of the larger coastal cities of Brazil brought a striking reduction in the observed incidence of the disease it did not lead to complete disappearance of infection from tributary areas Such disappearance had been expected from the experience gained in other Latin American countries The occurrence in 1929 of two widely separated outbreaks at isolated points at Socorro Colombia (Pena Chavarria Serpa and Bevier 1930) and at Guasipati Venezuela (Benarroch 1936) having no possible connection with each other or with the infected areas of Brazil caused more and more epidemiologists to question the validity of the key center theory

The failure to eliminate yellow fever from Brazil through antimosquito campaigns in the coastal cities led in 1930 to a radical change in the plan of campaign It was decided to extend control measures against the vector *A aegypti* to the smaller cities and towns in the areas contiguous to the large cities and to keep on cleaning up smaller and smaller towns and villages until the disease finally disappeared The first application of this plan called for mosquito control in all towns of 2 000 population and over which had previously been attacked by yellow fever in those districts of northeast Brazil lying between the São Francisco and Parnaíba Rivers The cleaning of this area long thought to be the last endemic focus of yellow fever on the American continent was considered an essential step preceding more detailed studies of the Amazon region to the north and the suspected areas to the south

The introduction at this time of a system for routine collection and examination of liver specimens from patients dying with acute febrile illnesses soon showed that a considerable number of deaths from yellow fever were regularly occurring in a silent endemic area of northeast Brazil in the absence of recognized outbreaks The collection of liver specimens also demonstrated that this silent endemic area was much more extensive than had been indicated by previously discovered outbreaks

It soon became apparent that aegypti control in the small towns and villages would not eliminate yellow fever from northeast Brazil even though the towns themselves were kept free of infection The viscerotomy service continued to identify fatal cases in rural areas Field investigation uncovered

an unexpected distribution of *A aegypti* in the rural homes a type
tribution not hitherto encountered in the Americas The phenomer
attributable to the low rainfall of the region which makes it necessar
rural householders to store as much rain water as possible for use in th
seasons *A aegypti* thus finds an abundance of favorable breeding plac
each home when it arrives in the egg or larval stage in the water jar
travelers The people of that region are unusually nomadic in habit
move from place to place in the dry seasons This may account for the ma
tenance of yellow fever in the area on an endemic basis

Following the discovery of the rural distribution of *A aegypti* the co
trol measures were extended to the houses of a large rural area includin
parts of Pernambuco and Ceará Yellow fever rapidly disappeared from th
region under control just as it had previously done from towns and cities
These happy results again gave rise to a hope that the Americas might be
freed of yellow fever by a thorough pursuit of *A aegypti* control Unfortu
nately the course of events elsewhere soon revealed the illusory nature of
this optimism

WEST AFRICA

The extension of the yellow fever investigation to West Africa a possible
endemic region was a part of the program outlined by the original Yellow
Fever Commission The aims of the commission were (*a*) to determine
whether the reported yellow fever of that region was yellow fever and (*b*) if
the presence of yellow fever was confirmed to ascertain whether control
measures were feasible The 1920 commission known as the Yellow Fever
Commission to the West Coast of Africa with General Gorgas at its head
as composed of Dr R E Noble Assistant Surgeon General United States
rmy Dr Juan Guiteras Director of Public Health of Cuba Dr Adrian
okes Assistant to the Professor of Pathology Trinity College Dublin Dr
E Horn of the West African Medical Service and Dr W F Tytler of
staff of the British Medical Research Council General Gorgas became
and died in London on July 4 1920 The commission with General
ble in charge arrived in Lagos Nigeria in July 1920 Here it established
quarters and opened a laboratory From this point the members car
their investigations into Nigeria Dahomey the Gold Coast Senegal
elgian Congo and Sierra Leone In December after 4 months of activ
e commission submitted its report to New York No authentic case of
fever had been encountered Conferences and a

tistics records, however, gave strong indication of the presence of the infection within recent years. The region of suspected infection was vast, travel difficult, and living conditions extremely primitive. The control of yellow fever, even under these trying conditions, was regarded as not altogether impracticable. The commission recommended that its report be accepted merely as a progress report, and that another commission be sent out, equipped for a more extensive and prolonged investigation.

It was not until 1925 that the situation in the Americas appeared secure *enough for the Foundation to release men to return to Africa to carry out the* recommendations made by the 1920 commission. Dr. Henry Beeuwkes was selected to direct the new investigations. Before the end of 1925, the staff of the new West Africa Yellow Fever Commission, consisting of a pathologist, three physicians experienced in yellow fever control, an entomologist, a sanitary inspector, an office assistant, and a laboratory technician, was well established in five prefabricated buildings brought from New York and erected in Yaba, a suburb of Lagos. From time to time other personnel were sent to join the commission in Nigeria.

Preliminary to control work in West Africa it was considered necessary (*a*) to learn the characteristics and epidemiology of the disease in West Africa and its relationship to the fever of the Western Hemisphere, (*b*) to attempt the isolation of the organism that causes the disease, (*c*) to discover the method of transmission, and (*d*) to identify those areas in which the disease is continually present.

Through the cooperation of the governmental authorities, the commission had an opportunity to study many cases of yellow fever and several epidemics in Nigeria as well as in the Gold Coast. Investigations were also undertaken in other countries. During the first 2 years members of the commission studied the bacteriology, pathology, and clinical aspects of numerous cases of yellow fever. Efforts to isolate *L. icteroides* from 67 cases consistently failed. A concerted search was then made for an animal susceptible to yellow fever infection. Finally success was attained when Stokes, Bauer and Hudson (1928*b*) were able to transmit yellow fever to monkeys by blood inoculation from a human case, first to *Macacus sinicus*, and then to the even more susceptible *Macacus rhesus*.

The discovery of a satisfactory laboratory animal made possible a period of highly fruitful research. Stokes, Bauer, and Hudson (1928*b*) were able *to prove conclusively that the causative agent of yellow fever was a* filtrable virus and not *L. icteroides*, as claimed by Noguchi, Sawyer, Kitchen, et al.

(1930), working in the newly established Yellow Fever Laboratory of the International Health Division in New York, obtained evidence by cross immunity tests in monkeys that the yellow fever in Africa, that in Brazil, and the historic yellow fever of Panama were the same disease. In order to pursue parallel studies on yellow fever in West Africa and in South America, another laboratory was established in Bahia, Brazil. This was placed under the direction of Dr. Nelson C. Davis.

Using the monkey as a test animal, it was quickly established that mosquitoes other than *A. aegypti* can transmit yellow fever virus efficiently by bite. Bauer (1928) reported from Africa that *Aedes luteocephalus* and *Aedes apicoannulatus* compared favorably with aegypti in their ability to transmit yellow fever virus. Davis and Shannon (1929*b*, 1931*a*) in Brazil obtained similar results with *Aedes scapularis* and *Aedes fluviatilis*. Davis (1930*a*) proved that the common cebus monkey of Brazil, when infected with yellow fever, has sufficient virus circulating in the blood to infect *A. aegypti* mosquitoes.

Another very useful laboratory animal became available when Theiler (1930*a*) discovered that white mice are susceptible to yellow fever virus if inoculated intracerebrally. An immunity test in mice, developed soon thereafter (Sawyer and Lloyd, 1931), was promptly introduced for field surveys. A small series of sera from children living along the upper Amazon River in Brazil and Peru was collected and tested in 1931 (Soper, 1937*a*). The results clearly indicated that some immunization was occurring in the complete absence of observed outbreaks of the disease.

The stage was now all set with the techniques for precise diagnosis and study of yellow fever in the field, and epidemiologists were in a position to take full advantage of the opportunities presented to them.

At about this time, 1930, a sharp outbreak of an acutely fatal disease occurred in Muzo, an isolated village in the department of Boyaca, Colombia (Soper, 1935*b*). The Muzo region had often been the scene of such epidemics. For a long period of years yellow fever had been suspected as the cause, but since *A. aegypti* was never found, even on repeated search by competent field workers, the diagnosis of yellow fever could not be finally accepted. With the development of the mouse protection test it was possible to obtain confirmatory information. Sera were collected in Muzo by Dr. Moreno Perez and forwarded to New York in 1931. The results of the tests on these sera in mice demonstrated clearly that yellow fever had been pres

Before the situation at Muzo could be fully studied a rural outbreak of yellow fever quite outside the known endemic region was discovered in the Valle do Chanaan, Espirito Santo, Brazil (Soper, Penna, et al, 1933) The local health officer, who was familiar with yellow fever, diagnosed a case as suspicious and carried out an autopsy when the patient died A positive diagnosis of yellow fever was made by pathologists in Rio de Janeiro and Bahia after examination of the liver sections from this case In the course of an epidemiologic investigation the diagnosis was confirmed by additional autopsies and by isolation of yellow fever virus in rhesus monkeys from a patient on the 2d day of his illness This investigation also failed to disclose any evidence of the presence of *A aegypti* in the area in which cases were occurring

Since the epidemic was apparently on the decline when discovered it soon disappeared from the community A subsequent immunity survey indicated that infection, although widely disseminated geographically, was limited to a surprisingly low percentage of those tested On this basis, the spontaneous disappearance of yellow fever in the area was attributed to inefficiency of the insect vector rather than to failure of the supply of susceptible humans

The finding of yellow fever without *A aegypti* came as a heavy disappointment to those who had believed so firmly that the man–aegypti cycle represented the only significant mechanism in the maintenance of yellow fever It was quite clear that the prevalent conceptions of the epidemiology of yellow fever would have to be revised Under these circumstances the program for banishing yellow fever from the earth through key center control obviously had to be abandoned

ATTEMPTS TO FIND A NEW BASIS FOR CONTROL

The true epidemiologic significance of yellow fever without *A aegypti*, or jungle yellow fever as it came to be called, was not fully recognized at the time of the first observed outbreak in Brazil, and the statement was made that This epidemic [Valle do Chanaan] was apparently self limited, and it is possible that there are no rural regions in America which are truly endemic for yellow fever" (Soper, Penna, et al, 1933) A representative of the Brazilian Yellow Fever Service visited San Ramón, an isolated village in Bolivia, in September 1932, and was told of suspect cases of yellow fever occurring as early as June of the same year A thorough survey of the com

munity failed to reveal larvae or adults of *A aegypti* but two of the three blood specimens taken at this time were shown to be protective against yellow fever Early the next year reports of other suspect cases were received and investigation indicated that two thirds of the population of the village had suffered from an illness resembling yellow fever Additional positive blood specimens were secured and a liver specimen from a fatal case soon confirmed the presence of yellow fever

These instances of unexpected behavior on the part of the yellow fever virus made it obvious to all that the old concepts of yellow fever had been far too limited largely due to the lack of laboratory methods for field studies and that new knowledge had to be sought upon which to build a satisfactory understanding of yellow fever epidemiology

Fortunately the tools necessary for digging out the data upon which to base the new epidemiologic concepts of yellow fever were now at hand and eager workers were available to apply these tools The most urgent problem appeared to be that of determining the distribution of yellow fever throughout the world The methods found most valuable for this study were (*a*) the mouse protection test which could be applied to samples of blood from selected groups of the population in the areas to be investigated (*b*) the routine post mortem examination of liver tissue (*c*) the isolation of the virus from human beings or animals infected in nature Methods (*a*) and (*b*) are independent of the notification of suspect cases of yellow fever The information secured by each of these methods is complementary to that secured by the other The immunity survey gives a cumulative picture of past exposure to the virus of yellow fever the diagnosis of cases by liver examination gives a current picture of mortality from yellow fever and an indication of the present distribution of the disease The isolation of virus in laboratory animals is the most conclusive method of all for definite diagnosis of a case but is of limited use in distribution studies

IMMUNITY SURVEYS

The observation that yellow fever conferred a lasting immunity on those who recover from an attack had been well established by physicians and by the experiences of settlers and sailors over the last three centuries The present investigators now needed a practical laboratory test capable of demonstrating this immunity so that they could make a retrospective diagnosis of yellow fever Shortly after the discovery of the susceptibility of

rhesus monkeys to yellow fever Stokes Bauer and Hudson (1928*b*) showed that a small dose of serum from a convalescent animal protected the monkey against an inoculation of virulent yellow fever virus A protection test for routine demonstration of specific antibodies in sera was quickly worked out

The first yellow fever immunity survey was made in Nigeria by Beeuwkes Bauer and Mahaffy (1930) As it was thought necessary to use two rhesus monkeys for each serum tested the number of blood specimens that could be examined was extremely limited and only a few communities could be studied

The important discovery by Theiler (1930*a*) that white mice are susceptible to yellow fever when inoculated with the virus intracerebrally made possible a wider application of the protection test to field studies Sawyer and Lloyd (1931) developed a practical protection test in mice a mixture of the serum and a suspension of virus containing mouse brain is injected intraperitoneally while at the same time a small quantity of sterile starch solution is injected into the brain of the anesthetized animal to localize the virus there

With the cooperation of the governments concerned the International Health Division began in 1931 to apply this mouse test in a widespread survey of the geographic distribution of immunity to yellow fever From many parts of the world sera were collected for testing in the yellow fever laboratories of New York Rio de Janeiro Bahia Bogotá Entebbe and Lagos In round numbers between 1931 and 1949 a total of 95 000 human sera were examined (Table 30)

The methods used in testing the sera were those originally described by Sawyer and Lloyd (1931) except that a 20 per cent suspension of virus-containing mouse brain was generally employed instead of the 10 per cent recommended in the original technique The increase in the amount of virus made the test less sensitive and thus diminished the chance of false positives If a serum protected all or all but one of six mice it was concluded that the donor had been infected with yellow virus at some time in his life On the basis of interest and accessibility representative towns and villages were selected for detailed investigation Blood was taken from about 50 persons in each place 25 adults and 25 children selected at random without regard to previous illnesses Sometimes the full number of suitable specimens could not be secured Great care was taken to select only donors who had never been out of the locality Age sex race tribe and other particulars regarding the donors were recorded on a standard form In some in

stances in regions in which it was thought that yellow fever had never be... present all the specimens were taken from adults in order to obtain much information as possible regarding the yellow fever history of th... region Where there had been known epidemics specimens sometimes wer... taken from children only to obtain the maximum amount of information about recent yellow fever

By the procedure established the blood was drawn from a vein of the arm into vacuum syringes of the venule type with a capacity of 30 cc Usually the specimen was sent to the nearest local laboratory for separation of the serum and then shipped under refrigeration to the laboratory in which the tests were to be made When the blood was collected at a great distance from a laboratory it was found advisable to draw off the serum in the field into a second venule of smaller capacity (usually 10 cc)

By classifying the donors according to age in relation to immunity it was sometimes possible to construct roughly a yellow fever history of the community and even to establish approximate dates of outstanding epidemics The impracticability of bleeding very young children however often made it difficult to obtain data on the presence of yellow fever during the previous 5 or 10 years

As immunity against yellow fever was found to be more widely distributed in Africa and South America than had been expected the specificity of the test soon came into question It seemed advisable as a control to make tests of human sera from countries believed to have been always or for generations free from yellow fever Eight hundred and seventy six specimens of blood were therefore collected from Australia Ceylon China Java India the Malay States the Philippine Islands and Syria Only two specimens of these sera gave protection against yellow fever virus Both of these came from India and as far as could be learned neither of the donors had ever been exposed to yellow fever Among 481 sera from Italy Spain Portugal ...anada and the United States where yellow fever was formerly present ...it is now absent only one was protective (Sawyer Bauer and Whitman ...37)

Sera from 10 African countries without history of yellow fever except ...sibly brief local introductions at ports but on the same continent with a ...e infected area gave protection in seven out of 850 instances or 0 8 per ...

The countries included in this group were Morocco Egypt Kenya ...anyika Abyssinia Zanzibar Southern Rhodesia Bechuanaland Pro...ate and the Union of South Africa (Sawyer and Whitman ...

The few positive results less than 1 per cent in countries believed to have been free from yellow fever are possibly due to several sources of error including inaccurate information from the donor as to previous opportunity for exposure technical errors in the performance of the test or some nonspecific protective substance in the blood

The general negative results tended to confirm the reliability of the large number of positive results obtained in other regions

Africa The systematic immunity survey of Africa began in Nigeria in 1931 The results for the greater part of West Africa were published in detail by Beeuwkes and Mahaffy (1934) and those for the French Cameroons French Equatorial Africa the Belgian Congo and Angola by Beeuwkes Mahaffy et al (1934) The remainder of West Africa consisting of a large part of French West Africa was surveyed independently for the French government by Stefanopoulo of the Pasteur Institute in Paris These results were published by Boye (1933) Stefanopoulo used an intracerebral protection test in mice in the sera he collected in West Africa In his later tests of sera from North Africa and Madagascar in uniformity with those of other investigators he employed the intraperitoneal test in mice Results from other countries of Africa were obtained by Sawyer and Whitman (1936) through examination in New York of sera collected and forwarded by health officials of the various governments

Additional information regarding two of the countries included in the regional surveys has been published Hewer (1934) who had collected sera from the Anglo Egyptian Sudan summarized and discussed the protection test results for that country Mouchet van Hoof et al (1934) who had co-operated in the immunity survey of the Belgian Congo presented further particulars regarding the conditions there

These surveys showed a marked inconsistency between the reported distribution of cases of yellow fever and the distribution of demonstrable immunity to the disease In a region extending from the coast of Senegal eastward for approximately 3 300 miles to the upper reaches of the White Nile in the Anglo Egyptian Sudan immunity was found to be widely but irregularly distributed On the north this region is limited by the Sahara Desert On the south the boundary follows the coast of the Atlantic Ocean from Senegal to the extreme northern part of Angola and then runs eastward across Angola and the southern part of the Belgian Congo The region has a maximum width of about 1 400 miles and lies between latitude 16 north and 6 south (Sawyer and Whitman 1936)

Human blood sera collected in localities scattered throughout the parts of Africa lying outside the region outlined above were negative in the mouse test except in a few rare instances The chance of finding an isolated individual with protective blood seemed to rise with the number of sera collected in a locality and with the nearness to the area of high immunity

Within the region of immunity there were areas in which no immune persons were found others in which only a small proportion of the tests on adults were positive It was concluded nevertheless that the region as a whole ought to be considered as endemic in the sense that yellow fever is always present and widely distributed To what extent it is continuously present in any place that showed a high prevalence of immunity but had no known history of yellow fever is difficult to determine either constant endemicity or a recent sharp outbreak might have produced the observed conditions Likewise the places in which only a few adults were immune might either have undergone an epidemic many years earlier or have remained in a constant state of low endemicity under conditions that make infective exposures infrequent and limited mainly to the adult population

The African region of immunity may be divided into two parts The western area extends to the eastern border of Nigeria and includes also the coastal region from Nigeria to Angola The remainder of the region is the eastern area

Numerous epidemics of yellow fever both on the coast and in the interior have been described in the western area All of the historic outbreaks of yellow fever in Africa have taken place within this area except for two invasions of the disease in northern Morocco in 1801 and 1881 and some epidemic appearances of yellow fever in the coastal towns of Angola up to 1899 (Sawyer and Whitman 1936)

In the eastern area the situation is different Yellow fever had never been recognized except for a probable case recorded by Hewer (1931) in the Anglo Egyptian Sudan during the course of the immunity survey Europeans stationed in areas where a large proportion of the natives are immune have never been known to contract the disease The heavily immunized areas therefore appear to be continuously endemic rather than epidemic as any severe outbreak would almost certainly have been noticed

Within the eastern area there is a zone of high prevalence of immunity among children and adults The number of places sampled was not great enough to delimit clear boundaries for this zone It lies between latitudes 3 and 8 no[illegible]

across French Equatorial Africa overlapping the northern edge of the Belgian Congo and into the Anglo Egyptian Sudan To the north and south of this zone the incidence of immunity diminishes

The environmental *conditions that favored yellow fever in this zone of* high prevalence in the eastern area were in great part unknown To investigate this situation further The Rockefeller Foundation in 1936 at the invitation of the British Colonial Office with close cooperation and financial assistance from the Uganda government and later from the other governments of British East Africa established the Yellow Fever Research Institute at Entebbe Uganda

In 1941 Hughes Jacobs and Burke reported the results of mouse protection tests on 3 911 specimens of sera collected in 1936–1937 from 49 different localities in the Uganda Protectorate The results of these tests showed that eastern central and southwestern Uganda had probably been free of yellow fever infection In the northern and western portions of the protectorate there was a low incidence of immunity which did not reflect recent presence of infection with the virus These regions adjoin areas outside Uganda where the incidence of yellow fever immunity was known to be high In only one part of Uganda the Bwamba forest area of the Toro District which geographically represents a continuation of the forest of the Belgian Congo was there cause to suspect a continuing yellow fever infection possibly endemic in character This suspicion was subsequently borne out by the occurrence of proved outbreaks of yellow fever in both the human and monkey populations (Mahaffy Smithburn et al 1942 Haddow Smithburn et al 1947)

From 1937 to 1943 Mahaffy Smithburn and Hughes (1946) continued the immunity survey of Central and East Africa During that period 10 274 sera from residents of 10 countries were examined in the Entebbe laboratory In this report are included the results of all tests carried out at the institute in Entebbe although some of those with sera from Uganda the Anglo Egyptian Sudan and the Belgian Congo had already been reported (Kirk 1941 Findlay Kirk and MacCallum 1941 Hughes Jacobs and Burke 1941 Liegeois 1941) The intraperitoneal protection test was used throughout The 20 per cent suspension of virus containing mouse brain was employed in the earlier tests but this was later reduced to 10 per cent and from the beginning of 1943 to 1 per cent A satisfactory test in baby mice was used during the year *1943 for sera available in amounts less than* 3 cc On the basis of experimental evidence these modified techniques were

considered more sensitive for detection of antibody in low titer than the standard intraperitoneal method and no less specific than the latter

The results of this survey confirmed earlier findings in Central and East Africa Recently infected areas in which yellow fever was not previously known to have existed included Eritrea western Somalia central Kenya and the Balovale district of Northern Rhodesia

That clinical cases of yellow fever had never been recognized in these territories need not be surprising The protection test provided the first indication that the disease had occurred in the Anglo Egyptian Sudan General recognition of cases came later when the most extensive epidemic ever recorded in any part of Africa was observed in the Nuba Mountains (Kirk 1941) The difficulty of finding and diagnosing yellow fever in the native African is well illustrated by the experience of the Entebbe group in Bwamba County Here again the protection test indicated that the disease had been present within recent years but the most careful search by experienced workers failed to reveal a single case during a 4 year study It was only after the protection test demonstrated the existence of the disease in a restricted area within recent months that cases were found The explanation for this lies in part at least in the fact that a high percentage of yellow fever cases in Africans are so mild that they cannot be diagnosed clinically

Severe and fatal cases of yellow fever exhibiting the classic symptoms of the disease do occur in Africa but apparently they are rare Under the conditions prevailing there the fact that such cases have not been recognized does not constitute a sound basis for declaring an area free of the infection The assumption that where yellow fever has occurred it can and may occur again makes it reasonable for the purposes of control to regard the whole area in which immunes are found as the endemic zone of Africa

From 1944 to 1948 additional immunity surveys were carried out in Kenya Tanganyika and Northern Rhodesia and selected sera were tested from previously unsampled areas of Nyasaland Southern Rhodesia Mozambique Bechuanaland and Swaziland Some tests were also performed on sera from residents of Egypt Arabia and Somaliland In 1949 Smithburn and his associates (Smithburn Goodner et al 1949) reported the results of tests on 5 151 sera from persons indigenous to these areas These results showed that yellow fever had occurred much farther to the south in Africa than had previously been supposed The southernmost locality in which immunes were found was Tsau in Bechuanaland which lies just south of the 20th parallel Since surveys to the south of this locality have been very

meager there is no reason for assuming that the southern limit of the yellow fever zone of infection in Africa has yet been found

Among the countries surveyed the incidence of immunity to yellow fever was highest in Northern Rhodesia and within that country the incidence was greatest along the Zambesi River Immune individuals were found in every country sampled except Arabia and Swaziland

Tests of 138 specimens from nine localities in Egypt gave satisfactory results although many of the sera had been contaminated by bacterial growth One of 72 specimens taken from children and four of 366 from adults were protective Not more than one protective serum was found in any one locality Presumably none of the donors had been vaccinated or had traveled far from home Nevertheless in view of the poor quality of the sera further studies in Egypt must be undertaken before definite conclusions can be drawn

In 1949 additional human sera were collected from seven villages from the Lake Ngami region of Bechuanaland These sera were tested in mice against the 17D strain of yellow fever virus at the South African Institute for Medical Research in Johannesburg by Dr J H S Gear who kindly made the results available to the International Health Division Of 131 sera five gave positive protection test results and 30 gave inconclusive results So far no *positive sera have been obtained in South Africa apart from those due to* previous vaccination Surveys are now being planned along the northern frontiers of South Africa to delineate the southern limit of the endemic zone of yellow fever

South America The systematic immunity survey in South America began in 1931 With the exception of Argentina and Uruguay it was extended to all countries on the continent During the early stages since nothing was known of the existence of jungle yellow fever the collection of blood specimens was limited to towns and cities Later on, as understanding of jungle yellow fever increased the survey was also extended to rural areas In each community the aim was to obtain specimens from 100 persons born and continuously resident in that place one half from persons under 10 years of age Not more than two persons from any one family were to be bled one under and the other over 10 years of age In practice these directions were difficult to carry out Collections were often made in schools and full information regarding the history of the children was not obtainable In some localities especially among the Indian tribes only an approximate age could be elicited In others population movement was so great because of

migratory labor seasonal floods and occasional droughts that very few families had been constantly resident in one district throughout their lives In spite of these difficulties however it is believed that the survey data give a good general idea of the yellow fever situation in the study areas

The classification of the population groups as urban or rural is somewhat arbitrary and not entirely satisfactory The urban group includes many persons living in small villages and settlements as well as those in organized towns and cities The rural group includes those living in both heavily and sparsely populated agricultural districts as well as those from jungle areas

The results of the survey up to 1935 were reported by Soper (1937a) (Table 30) The general conclusion was that although visible urban and maritime outbreaks of yellow fever may decline and even cease entirely for a time there is a vast silent reservoir of infection in the interior of South America Yellow fever infection transmitted by *A aegypti* has in the past been much more widespread in the interior of northeast Brazil than previously suspected even by investigators who had long kept the area under special observation Endemic areas of yellow fever instead of being limited to the coast of northeast Brazil extend to all of Brazil except a few of the southern states to Bolivia Peru Ecuador Colombia Paraguay and Venezuela The widely varying percentages of immunes in proved endemic regions depended to some extent upon whether transmission was due to *A aegypti* or to some other vector Much of this endemicity revealed a persistence of the disease in sparsely populated jungle districts where there were no aegypti mosquitoes That all the immunity was not due to infection with mild attenuated strains of the virus is amply proved by the fact that fatal cases were confirmed by liver examination in all but a few of the Brazilian states in Bolivia Paraguay Peru Venezuela and in the Magdalena and Orinoco Valleys of Colombia during the course of this survey Concurrent studies have not only borne out the results of the immunity survey but have also uncovered the existence of jungle infection in a number of places not indicated by the earlier surveys of the towns Apparently the negative results for the Guianas meant only that the disease had not invaded the cities in recent years

Those conducting immunity surveys found that the interpretation of results must be made with caution unless adequate samples of the population are tested In cities and towns where yellow fever is transmitted by the domestic mosquito *A aegypti* the ratios of exposure should be equal for males and females with possibly even more females than males attacked by

TABLE 30

IMM UNITY SURVEYS

Coun y	Area o a y u ban o u al)	Num ber of a as	Yea of urvey	No of p m n examined — To l — Spe	To l — Po ve	0 14 yr — Sp	0 14 yr — Po	5 y + — Sp	5 y + — Po ve
E rope and North Amer ca									
I a y	U	3	932	64	0			64	0
Spa n	U	2	1932	42	0			42	0
Po ugal	U	1	1932	43	0			43	0
Canada	U	1	1932	109	1			109	1
Un d a es	U	5	1932	223	0	61	0	162	0
As a A a M nor and A stral a									
Cey on	U	1	932	44	0			44	0
Ch na	U	1	1932	1 8	0			1 8	0
Java	U	2	1932	49	0	2	0	47	0
India	U	0	1932	492	2	87	0	405	2
Ma aya	U	1	1932	52	0			52	0
Ph pp nes	U	1	1932	27	0			27	0
Sy ia	U	1	1932	60	0	15	0	45	0
Au a a	U	1	19 2	34	0			34	0
W e t Ind es									
Ba bado	U	1	1932	73	0	42	0	31	0
Cuba	U	6	1932	504	4	322	0	182	4
Jamaica	U	3	1932	156	15	75	0	8	5
Pu o R o	U	5	1932	197	9	14	0	18	9
S Lu a	U		1932	63	0	46	0	17	0
T n d	U	4	1932	184	2	158	0	26	2
Central Am r a									
B h Hon u a	U	2	1932	94	11	34	1	60	10
	R	4	1941	2 2	3	9	0	21	3
Cos a R a	U	5	1932 34	190	18	69	0	121	18
	R	17	1939–40	396	0	190	0	206	0
El Sa ad	U	4	1932	195	36	86	2	09	34
	R	5	1941	241	11	22	0	1 9	11
Gua ma a	U	4	1932	184	21	67	0	117	21
	R	6	194 –42	343	7	174	0	69	7
Iond as	U	3	1932	141	8	55	0	86	18

Note U = u ban R = u al.

TABLE 30 (*Continued*)

IMMUNITY SURVEYS

Central America (Continued)

Country	Area or locality (urban or rural)	Number of areas	Year of survey	No. of specimens examined: Total		0–14 yrs		15 yrs +	
				Spec	Positive	Spec	Positive	Spec	Positive
Mexico	RU	20	1932	1 089	191	707	36	382	155
	RU	18	1932 34	953	192	587	34	366	158
	R	12	1942	698	23	416	0	282	23
			1942	806	27		0		27
Nicaragua	U	3	1932	182	41	92	4	90	37
	R	4	1941	234	0	107	0	127	0
Panama	U	3	1932	196	21	49	0	147	21
	RU	8	1936–37	328	21	212	3	116	18
	R	46	1941 42	1 923	88	1 353	21	570	67
	R	28	1949	835	160			835	160

South America

Country	Area or locality (urban or rural)	Number of areas	Year of survey	Total Spec	Total Positive	0–14 yrs Spec	0–14 yrs Positive	15 yrs + Spec	15 yrs + Positive
Bolivia	RUI	30	1932 35	971	288	550	89	421	199
Brazil	U	1	1929	(pt) 76	42	12	8	64	34
				(cf) 250	105	54	26	196	79
	U	1	1931	(pt) 555	329	149	76	406	253
				(cf) 595	129	145	29	450	100
	R	36	1930–35	3 684	888	1 893	225	1 791	663
	U	164		13 947	3 006	10 273	1 392	3 674	1 614
	?	3		394	71	365	51	29	20
	I	2		175	63	34	7	141	56
Chile	U	2	1934	119	0	119	0		
Colombia	R	1	1931 32	88	41	14	0	74	41
	U	21	1932	1 017	100	874	48	143	52
	R	15	1932 35	668	121	326	11	342	110
	U	18	1932 35	797	41	563	3	234	38
	R	77	1935–42	4 320	889	1 890	137	2 430	752
	U	53	1932–41	1 998	86	1 268	20	730	66
	U	5	1932	258	1	258	1		
Ecuador									
Amazon Valley	R	6	1935–36	248	33	115	10	133	23
Pacific Slope	U	9	1933 34	549	0	549	0		

Note I = Indians pt = protection test, cf = complement fixation

TABLE 30 (*Continued*)

IMMUNITY SURVEYS

South America (*Continued*)

Country	Area or locality (urban or rural)	Number of areas	Year of survey	No of specimens examined: Total		0-14 yrs		15 yrs +	
				Spec	Positive	Spec	Positive	Spec	Positive
British Guiana	U	4	1934	192	4	122	0	70	4
	R	1	1938	46	6	2	0	44	6
	R	10	1938	289	126	8	2	281	124
	R	1	1939	14	11				
	R	9	1940	217	59	84	15	133	44
Dutch Guiana	U	4	1934	200	4	167	0	33	4
	U	1		293	59				
	R			76	12				
French Guiana	U	3	1934	184	5	111	0	73	5
Paraguay	U	4	1932	179	1	142	0	37	1
Peru									
Amazon Valley	RU	12	1933 34	604	165	349	37	255	128
Pacific Slope	U	12	1934	857	4	851	3	6	1
Venezuela	U	50	1934–35	2 233	47	2 170	46	63	1
	R	2	1934 35						

Africa

Country	Area or locality (urban or rural)	Number of areas	Year of survey	Total Spec	Total Positive	0-14 yrs Spec	0-14 yrs Positive	15 yrs + Spec	15 yrs + Positive
Abyssinia	RU	7	1941 41	223	1			223	1
Algeria	U	1	1934	28	0			28	0
Anglo-Egyptian Sudan	RU	12	193? 34		67		7		60
	RU	14	1933 34	575	65	243	7	332	58
	RU	6	1935 36	158	61	25	3	133	58
Nuba	RU	29	1937 38	514	165	106	31	408	134
Fung	RU	11	1937–38	163	19	23	0	140	19
	RU	52	1938 42	1 262	174	568	40	694	134
	RU	3	1941	64	44	12	7	52	37
	RU	6	1941	570	4			570	4
gola	RU	19	1933 34	949	11	475	4	474	7
bia	U	2	1944	36	0	7	0	29	0
huanaland	U	1	1933	13	0			13	0
	RU	7	1945	293	8	144	2	149	6

TABLE 30 (*Continued*)

IMMUNITY SURVEYS

Africa (*Continued*)

Country	Area or locality (urban or rural)	Number of areas	Year of survey	No. of specimens examined					
				Total		0-14 yrs		15 yrs +	
				Spec	Positive	Spec	Positive	Spec	Positive
lgian Congo	RU	5	1932 33	258		70		188	
	RU	43	1933 34	1 740	154	693		1 047	
	RU	136	1938–41	7 083		3 399		3 688	
	R	1	1939	26	0			26	0
	RU	83	1939–41	2 611		1 420		1 191	
	RU	60	1940–41	1 626	105	1 218	61	378	44
iti h Somal land	U	3	1934	44	0			44	0
homey	RU	5	1932	188	56	148		40	
ypt	U	4	1932	237	3	15	0	222	3
	RU	9	1944	438	5	72	1	366	4
trea	RU	15	1942–43	526	21	287	14	239	7
hiopia	U	1	1933	27	0			27	0
ench Cameroons	RU	5	1932 33	250	14	123	3	127	11
	RU	9	1933–34	496	18	297		119	
ench Equator al Afr ca	RU	32	1932 33	1 298	263	738	63	560	200
	RU	37	1933–34	1 643	303	865		778	
ench Sudan	RU	2	1932	130	0	75	0	55	0
mbia	RU	2	1932	68	18	54		14	
	RU	5	1935	225	62		14		48
ld Coast	RU	35	1930–33	861	168	743		118	
enya	RU	4	1933 34	153	1	5	0	148	1
	RU	7	1940–43	564	3	274	1	290	2
	RU	5	1944–48	164	10	2	0	162	10
ler a	RU	5	1932	96	6			96	6
adagascar	RU	1	1934	20	0			20	0
o occo	U	3	1933	86	0			86	0
ozambique	RU	4	1946–47	68	1	6	0	62	1
iger	RU	11	1932	481	104	337		144	
igeria	RU	6	1929	340	56				
	RU	121	1931 34	5 607	1 508	3 149		2 458	
	U	1	1941	100	11	100	11		

TABLE 30 (*Continued*)

IMMUNITY SURVEYS

Africa (*Continued*)

Country	*Area or locality (urban or rural)*	*Number of areas*	*Year of survey*	*No. of specimens examined*					
				Total		0–14 yrs		15 yrs +	
				Spec	*Positive*	*Spec*	*Positive*	*Spec*	*Positive*
Nyasaland	RU	8	1935	8	0			8	0
	RU	19	1944–46	382	15	126	2	256	13
Portuguese Guinea	RU	32	1943–44	202	87	62	21	140	66
Rhodesia North	RU	4	1933	96	2			96	2
	RU	5	1941–43	298	15	79	2	219	13
	RU	10	1944–47	3 055	229	789	34	2 266	195
Rhodesia South	R	1	1933	22	0			22	0
	RU	10	1946–48	142	1	40	0	102	1
Sierra Leone	U	1	1929	34	1	34	1		
	RU	5	1932	149	19	97		52	
Somalia	RU	4	1942–43	146	5	15	0	131	5
	U	1	1944	36	3	14	0	22	3
Spanish Guinea	*RU*	*3*	*1935*	*99*	*10*	*8*	*1*	*91*	*9*
Swaziland		1	1945	46	0	14	0	32	0
Tanganyika	RU	8	1933	194	1			194	1
	RU	15	1941	467	1	221	1	246	0
	RU	12	1944–48	530	12	228	4	302	8
Tunisia	U	1	1934	25	0			25	0
Uganda	RU	12	1933–35	560	16	196	1	364	15
	RU	49	1936–40	3 892	152	2 229	25	1 663	127
				49	1				
	RU	56	1937–44	5 085	282	2 552	60	2 533	222
Union of South Africa	RU	3	1931	91	0			91	0
Zanzibar	U	1	1913	62	0			62	0
	U		1942	77	0			77	0

the mosquito assuming that mortality rates are equal in the two sexes equal percentages of immunes should be found for males and females Furthermore since transmission takes place within the home it may be assumed that the younger age groups are exposed *to infection* equally *with adults* whenever the disease invades the family circle In Brazil preliminary studies

(Soper and de Andrade 1933) on the distribution of immunity in the entire town of Cambucy immediately after a sharp outbreak of aegypti transmitted yellow fever confirmed this reasoning About 60 per cent of the population was immune the distribution of immunity being similar for males and females with high percentages of immunes among the younger age groups

The immunity picture in jungle yellow fever areas of infection is quite different Protection tests in the Valle do Chimaan where yellow fever without *A aegypti* was first demonstrated indicated that only 12 per cent of the population was immune The rates were especially low for both sexes in the younger age groups and for females in all age groups Immunity in males consistently increased with age during adult life Only under very primitive conditions in which all members of the family live in very close contact with the jungle do immunity rates attributable to jungle yellow fever approach with respect to age and sex those of the aegypti transmitted variety that is give a similar distribution among all age groups and both sexes

In towns afflicted with high aegypti densities the finding of reasonably high immunity rates in any age group *should* be taken as an indication that yellow fever has been present during the lifetime of that group Careful interpretation is necessary however if only occasional positives are found among younger people as in the towns of the Amazon Valley Negative results in the younger age groups in towns and cities with high aegypti indices *can* mean that the disease has not been widespread in those cities and towns during the lifetime of the negative groups But such an interpretation is not warranted in dealing with the problems of yellow fever in the tropical forest In areas of jungle yellow fever if the homes are not in intimate contact with the forest infections are limited chiefly to woodcutters and others who come in direct contact with jungle conditions

In considering the results of an immunity survey of a region the yellow fever investigators found that there is not necessarily a close correlation between the incidence of jungle yellow fever outside the towns and outbreaks of aegypti transmitted yellow fever Negative results from towns tell very little about conditions in the surrounding area

In immunity survey work general conclusions can be drawn *only* when high percentages of positives are found While low percentages cannot be disregarded they call for careful field investigation As Soper (1937*b*) pointed out The immunity survey is not a complete study of the situation in and of itself but it is an entering wedge which indicates where more intensive studies may be undertaken most profitably

Sneath (1939) published the results of immunity tests on blood sera from persons living or working in the sparsely settled areas of the interior of British Guiana In 289 specimens, 43 5 per cent possessed immune bodies to yellow fever The major foci of infection appeared to be in the Rupununi District near the Brazilian frontier The following year testing of 217 additional specimens confirmed the previous survey and made it clear that yellow fever was endemic in the hinterland of the colony (Sneath, 1940)

Schüffner, Walch Sordrager, and Hoekstra (1938*a* and *b*) reported the results of a yellow fever immunity survey in Surinam In the towns, 56 per cent of those tested had immunity to yellow fever, while among the Indians and bush Negroes the immunity rate was 16 per cent

From Colombia, Smith, Bevier, and Bugher (1943) reported results of further immunity surveys conducted between 1936 and 1942 A total of 6,318 sera from many different areas of the country were tested It was clear that immunity to yellow fever in Colombia was not limited to the areas in which proved cases had been discovered One of the most interesting results of this survey was the finding of sporadic immunes between 10 and 12 years of age in the rural zones near the mouth of the Atrato River near the Panamanian border The results also made it clear that jungle yellow fever is widespread in many areas in the lower valleys of the Colombian Andes, as well as on the llanos along the tributaries of the Amazon

Other Areas Outside of Africa and South America, investigations were made in as many other countries as seemed necessary to establish the general boundaries between the recently infected and the noninfected regions, and to find out where intensive local investigations were required

A total of 1,177 sera was tested from the following islands of the West Indies Barbados, Cuba Jamaica Puerto Rico, St Lucia, and Trinidad (Sawyer, Bauer and Whitman, 1937) Among them were 821 from persons under 20 years of age, none of whom gave blood serum that was protective against yellow fever virus Of 356 sera from adults over 20 years old, 30, or 8 12 per cent, showed immunity to yellow fever These results fit in well with the known history of yellow fever in that region

A total of 1,089 sera were tested from Mexico (Sawyer, Bauer, and Whitman, 1937) In all age groups except very young children the percentages of protective sera were high The fact that all sera from children born after 1925 gave negative results suggested that yellow fever had disappeared from the urban centers of Mexico about that time

A total of 1 182 specimens was tested from seven Central American countries (Sawyer Bauer and Whitman 1937) The results from El Salvador Guatemala British Honduras Honduras and Nicaragua were similar to those from Mexico The sera of persons under 20 years of age in Panama and Costa Rica gave no protection against yellow fever virus With two reservations it was concluded that at the time of the surveys yellow fever was not endemic on the American continents north of the Panama Canal However relatively few sera from young children were tested and as the specimens generally were collected in towns and cities it was not possible to exclude the possibility that some areas might harbor jungle yellow fever

To probe further into the question of yellow fever endemicity in rural areas in Central America Kumm and Crawford (1943) collected a total of 4 017 blood specimens from 85 localities in Mexico Panama and five other countries of Central America during the period 1939–1942 During recent years according to protection tests endemic yellow fever had been restricted to eastern Panama In Panamanian communities near the Colombian border mouse protection tests were positive in 27 children born after 1925 all but two of the children were Indians or mestizos living in the jungles about 120 miles from the Canal Zone Two immune persons were discovered in the jungle only 50 miles from the zone These findings agree with previously published data from Colombia in which positives were found near the Panamanian border

In January 1949 (Courtney 1950) the health officials in Panama were informed of five deaths of a questionable nature among natives living near the village of Pacora 18 miles east of Panama City Inquiry revealed that the men had died in November and December in the government hospital in Panama City All had contracted an acute febrile disease with symptoms resembling yellow fever After examination of liver specimens obtained from three of the men a diagnosis of yellow fever was made on all three and confirmed by experienced pathologists Field investigations disclosed other illnesses among natives in the area from which the fatal cases had come The situation was typical of outbreaks of jungle yellow fever in South America

Subsequent to this outbreak two additional deaths from yellow fever have been reported from Panama One was a young man from the mountainous district approximately three miles west of the village of Buena Vista on the Trans Isthmian Highway he died on September 10 1949 in the government hospital in Colon The other a Panamanian man aged 38 years

January 24 1950 also in the government hospital in Colon This man was probably infected in the rural district about five miles inland from Palmas Bellas on the Atlantic coast of Panama and represents the first case in many years known to originate west of the Canal Zone

To determine the distribution of immunity to yellow fever in Panama 835 human sera were collected in 287 localities from males 15 to 35 years of age who gave a history of either working or living in the jungle the results of the survey have been made available to the International Health Division by Dr K O Courtney coordinator of the yellow fever control program in Panama Of the 835 sera 19 per cent gave positive reactions in mouse protection tests Since these immune samples were from residents dispersed throughout the republic it was evident that yellow fever is a problem of serious dimensions for Panama

Immunity Surveys in Monkeys Since the discovery of jungle yellow fever in 1932 epidemiologists have taken increasing interest in the possibility that wild animals play a part in maintaining reservoirs of the virus Experience over the years has focused attention on primates especially monkeys and marmosets When they have recovered from an infection with yellow fever virus these animals usually become immune for life It is possible to capture them in their native habitats and then examine their blood to ascertain what proportion has been infected with yellow fever in the past

In epidemiologic studies on the type of yellow fever that occurs in the absence of *A aegypti* it has become evident that in many situations only persons who go into the jungle are exposed to infection This means that young children are less likely to show positive protection tests than are adults It is frequently not possible therefore to use the results of human immunity surveys to determine whether jungle yellow fever has been present in a region at a recent time Also during the past few years mass immunization campaigns in Africa and South America have produced a widespread immunity among the rural populations so that it will now be even more difficult to interpret the significance of positive tests

With monkey populations these two difficulties do not apply It is possible to classify monkeys fairly satisfactorily as to age by the state of dentition into (*a*) juveniles (*b*) subadults (*c*) adults (*d*) old If monkeys in the younger age groups are immune it is certain that yellow fever has been active in that area within a very few years It must be borne in mind however that the young of immune mothers have a passive immunity enduring for several

months There is evidence too that monkeys wander much less than h been supposed Each band has its own territory within the limits of whic it may be seen day after day traversing the same treetop paths and nightl returning to the same sleeping area (Gilmore 1943 Haddow Smithburn et al 1947)

The techniques and procedures used in Brazil for conducting surveys of immunity in monkeys were described by Gilmore in 1943 Many other studies have been carried out in connection with the epidemiologic investigations in selected areas Widespread surveys to determine the distribution of immunity to yellow fever in monkeys have been reported from Uganda by Haddow Smithburn et al (1947) and from Brazil by Kumm and Laemmert (1950) The first of these papers reported the results of tests on 150 monkeys collected over a period of several years in Bwamba County Uganda Immunity to yellow fever is widespread among the monkeys of the lowland forests No immune animals were found on the mountain slopes which lie to the east of the lowland area The incidence of immunity increases with age indicating that yellow fever is probably enzootic among the monkeys of the Bwamba lowlands

The survey of yellow fever immunity in monkeys has been extended during the past few years to southern and western parts of Uganda The results indicate a widespread incidence of infection among these animals throughout the forests in these areas

Bugher Macnamara and Hahn carried out between 1945 and 1948 a survey of yellow fever immunity in monkeys of various regions in Nigeria and the Cameroons The sera of 157 monkeys were tested and positives were found in all areas surveyed This was of especial interest in the highlands of the Cameroons as no evidence of yellow fever in humans had previously een obtained from that area In Brazil the sera of 5 857 monkeys and narmosets were collected over a period of 15 years from many different areas f the country (Kumm and Laemmert 1950) Every effort was made to cap ure the animals by trap but when this proved unsuccessful they were shot own from the trees and quickly bled from the heart to secure blood for a oratory test As was expected immune monkeys were present wherever re were records of fatal yellow fever infections in human beings n other places that had no past history of the existence of yellow fever ific immunity among local monkeys definitely established the presence e disease These areas are (a) in the far west of Mato Grosso near B

Esperidião (*b*) in Bahia west of the São Francisco River, and (*c*) near Ibirama Santa Catarina

As a result of the present outbreak of yellow fever in Panama a systematic monkey immunity survey has been undertaken in that country to determine the recent distribution of the infection Dr Courtney has informed the International Health Division that since 1948 over 442 monkey sera have been tested for yellow fever antibodies and 105 or 23 7 per cent have been found positive

It would thus appear that the monkey survey may now be considered as an important method for the discovering of new areas of yellow fever endemicity

VISCEROTOMY

The discovery of active yellow fever in the native populations of endemic areas is often most difficult Classic clinical cases of the disease occurring during epidemics among nonimmune troops or immigrants in endemic areas or in populations living beyond the usual yellow fever zone are not generally difficult to diagnose The diagnosis of yellow fever becomes rapidly more difficult however as the number of classic infections decreases Yellow fever may continue silently in a native population for an indefinite period The few fatalities are often attributed to malaria influenza typhoid fever or other acute infections Only through the use of proper laboratory methods can isolated cases observed in the field in endemic areas be diagnosed with certainty

The Americas By 1930 the Brazilian government was prepared to take drastic steps to obtain prompt reports of sporadic outbreaks and isolated cases in the silent areas Cases of yellow fever were still being identified in the interior of the northern states after infection had apparently been stamped out of the major coastal cities To help clarify this situation the state authorities of Pará granted permission in April 1930 for the Yellow Fever Service to perform partial autopsies on all fatal febrile cases of less than 10 days duration The third autopsy which was made on a child who had died in a village 3 miles from the city of Belem was positive for yellow fever Routine collection of liver specimens by physicians at autopsy was begun at Natal Rio Grande do Norte early in May 1930

It is interesting to note that the routine collection of liver specimens for the discovery of endemic areas of yellow fever was independently organized

ı north and south Brazil In May 1930 the yellow fever service of the State f Rio de Janeiro alarmed by the appearance of suspected cases in many arts of the state and recognizing the necessity of investigation developed n organization for securing liver specimens For this purpose several local ul registrars were brought together given demonstrations and instructions n the methods of obtaining liver specimens by partial autopsy and prom ed a cash payment for each specimen forwarded to the health department om a fatal fever case of less than 8 days duration presenting any of the ıore common symptoms of yellow fever In order to make the cooperation f these registrars effective all cemeteries were forbidden to permit burial f bodies unaccompanied by death certificates approved by the local regis ar About 100 specimens were examined by the State of Rio de Janeiro etween May and August 1930 To this state must be credited the first emonstration of the possibility of securing liver specimens from interior oints with nonmedical personnel (Soper Rickard and Crawford 1934)

The attempts to organize a practical service for the collection of liver pecimens in the State of Pernambuco led Dr E R Rickard to design an ıstrument later called the viscerotome for the removal of liver tissue ıthout autopsy (Rickard 1931) Early in 1931 viscerotomes were dis ıbuted to several towns of the interior of the state

The viscerotome is a sort of large trocar about 1 cm square with a slid ıg blade on one of its sides (Fig 65) There are cutting edges at the point o that no difficulty is encountered in puncturing the abdominal wall The uncture is made in the epigastric region the instrument being introduced vith the sliding blade fully closed so that only a single knife edge is pre ented Once the abdominal wall has been penetrated and the resistance of he liver is felt the sliding blade is retracted about 1 cm to open the forward nd of the instrument With the blade in this position the instrument is ushed through the liver until its point touches the ribs Then the sliding lade is pushed forward It too has a cutting edge so that a strip of tissue bout 1 cm square and more or less 6 cm in depth is cut out of the liver This tissue is enclosed inside the instrument which is then withdrawn

Once outside the body the sliding blade of the viscerotome is fully re racted With the probe the specimen of liver is removed from the instru nent and dropped into a small bottle containing 10 per cent formalin in hysiologic saline The incision in the body wall is then stuffed with cot on again using the probe It is not necessary for the person making the uncture to touch the cadaver or the specimen of liver The whole pro-

cedure takes no more than a minute After the bottle has been properly labeled it is ready for shipment to the central laboratory

The pathology of the liver in yellow fever was well known by the time viscerotomy was introduced Councilman (1890) studying yellow fever tissues at the request of Sternberg had described accurately the scattered

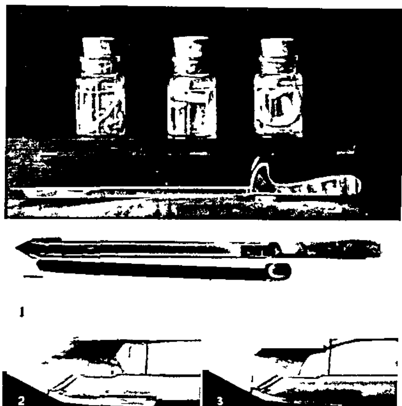


FIG 65 The viscerotome Above liver sections removed from three cadavers, and lateral view of the viscerotome Below enlarged details of the viscerotome

acidophil hyaline degeneration of parenchymal cells and parts of cells in necrotic areas of the liver, which we now know is characteristic of yellow fever

Da Rocha Lima (1912*b*) of Brazil, was responsible for calling attention to the characteristic changes of the liver on which a confident diagnosis of yellow fever may be based He described the division of the lobule into three zones central, midzonal, and peripheral The highest concentration of necrotic cells is to be found in the midzone, and the greatest accumulation

of fat occurs in the central and peripheral zones The necrotic cells ar
granular and have a peculiar hyaline appearance (Councilman bodies)

More recently Villela (1941) has described the classic picture of yellow fever in sections of human liver It is believed by pathologists who have made a special study of the subject that the yellow fever lesion when fully typical is characteristic and pathognomonic

On occasion when pathologists disagree in their findings on suspect liver specimens a full investigation in the field is necessary to clarify the diagnosis

Viscerotomy was not introduced to aid health officers in diagnosing suspect cases of yellow fever Such cases when fatal should be autopsied The purpose of viscerotomy is to discover fatal yellow fever cases in otherwise silent endemic areas It depends for its success upon establishing a routine systematic collection of liver tissue from all persons dying within 10 days after the onset of a fatal illness

Viscerotomy gives definite information that at a certain place on a known date someone died of yellow fever Negative findings are also of interest The absence of positive livers for a long enough time over a wide enough area results in the accumulation of a volume of negative evidence of great epidemiologic value (Soper 1936a)

In Brazil several types of viscerotomy organizations have been tried out with varying results under different conditions It was found advantageous to have full time viscerotomists in some of the larger cities although the service was suspended after several years of experience had shown that such cities produced no locally infected cases of yellow fever

As viscerotomy posts have been installed in many smaller communities where there are no doctors pharmacists have frequently served as represent atives In selecting a representative interest intelligence education and courage are more important than profession

In Brazil the success of the Viscerotomy Service is due principally to the roper selection and instruction of representatives who are to obtain speci nens the full application of existing legislation in regard to the registry of aths and the burial of bodies the provision of representatives to review death certificates before burial may take place the legalization of oblig ry viscerotomy and most important of all frequent visits to represent es by medical personnel of the Yellow Fever Service to maintain close tact between the service offices and the representatives

epresentatives are not considered employees of the service Rather they on the basis of a written agreement whereby the service agrees to p

a fixed sum for each suspect liver specimen plus an additional premium for the first liver found positive for yellow fever in any locality In return the representative agrees to follow instructions in obtaining specimens from cases that may be indicated by the service and to review and remit to the service all certificates of death filed in the locality

The registration of deaths and the supervision of burial places in Brazil is a function of the various state governments A death certificate may be signed by any licensed practicing physician or in the absence of such a physician by any responsible person who is acquainted with details concerning the death Before burial may take place the certificate must be presented to the local registrar who after registry makes out a burial permit In many regions because of the lack of practicing physicians the majority of certificates are made out and signed by the registrar himself When the Viscerotomy Service was organized the state departments of health agreed to modify the death certificates so as to include an additional section for the use of the viscerotomists In this section were provided spaces for noting the duration of illness of the deceased as well as the hour and date the deceased was seen by the viscerotomist Registrars were ordered by the state authorities to issue no burial permits without first obtaining the signature of the viscerotomy representative This allowed the representative to interview personally some relative or friend of the deceased to ask detailed questions about the illness and to make arrangements for viscerotomy if indicated

In connection with its valuable function of diagnosing yellow fever the Viscerotomy Service accumulated thousands of negative specimens that served to indicate the probable absence of the virus in many communities Much useful information regarding the distribution of other diseases producing characteristic lesions in the liver such as malaria schistosomiasis leishmaniasis and histoplasmosis has also been obtained by the service *(Davis 1934a Penna 1931 Villela 1943 Gast Galvis 1947 Para 1946)*

The tissues received from viscerotomy representatives include a small proportion of specimens unsuitable for examination In 2 to 3 per cent of the total specimens received post mortem changes are too far advanced for reliable diagnosis Occasionally also a puncture is unsuccessful and tissue other than liver is obtained Unscrupulous representatives whose compensation is based on the number of specimens forwarded have sometimes sent in multiple blocks of tissue from the same case under different

names and even blocks of tissue from animal livers Repeated fraud usually leads to detection in the laboratory

The growth of the Brazilian Viscerotomy Service was rapid between May 1 1930 and June 30 1933 a total of 29 593 specimens of livers from persons who died in central or northern Brazil was examined at the yellow fever laboratory in Bahia (Davis 1934a) By 1937 there had been established approximately 1 500 viscerotomy posts providing diagnostic service to a large area of Brazil (Rickard 1937) At the end of 1949 1 349 posts were functioning (Fig 66) The total number of liver specimens examined in the central yellow fever laboratory since the beginning of the service was 411 717

In 1932 following the successful inauguration of the Viscerotomy Service in Brazil the program was rapidly extended to the adjoining country of Bolivia and shortly afterward to Peru At the end of 1949 Bolivia had 69 viscerotomy posts which submitted approximately 500 liver specimens per year Peru where the yellow fever problem is limited to the eastern side of the Andes had collected a total of 1 679 liver specimens from 22 viscerotomy posts up to the end of May 1950

Colombia has a serious public health problem in jungle yellow fever and next to Brazil has made the most extensive use of viscerotomy Beginning in 1934 the number of viscerotomy posts grew continuously by early 1940 there were 117 which had submitted a total of 5 000 liver specimens (Gast Galvis 1941) By 1945 200 posts were in existence and a total of 22 000 liver specimens had been submitted (Gast Galvis 1945) From these 352 cases of yellow fever were diagnosed During the first 2 years of the olombian service all liver specimens were forwarded for examination to e laboratory of the Yellow Fever Service in Brazil With the founding of a llow fever laboratory in Bogotá in 1936 all specimens were examined illy although consultation with the Brazilian laboratory was sought on icult and doubtful cases By the end of March 1950 a total of 31 515 liver imens had been examined in Colombia

ther Latin American countries have made valuable though more spo use of viscerotomy Venezuela now has 75 posts which have collected al of 2 511 liver specimens In Panama due to difficulty in communica and perhaps other reasons viscerotomy has never functioned well se of the present outbreak of yellow fever there another attempt is be de to put this diagnostic service on an effective basis

Africa In Africa the endemic yellow fever areas are for the most part in colonial possessions Local conditions have made it difficult to introduce

FIG 66 Location of the 1 349 viscerotomy posts in Brazil at the end of 1949

viscerotomy successfully The directors of the yellow fever laboratories in Lagos Nigeria and Entebbe Uganda have made serious but fruitless efforts to do so during the past 15 years Some of the difficulties are reported below

Control

According to Bugher

In West Africa there is no attempt to certify deaths or to control burial save in a few very specialized localities. There is thus no way of compelling observance of any regulations established with regard to viscerotomy. Since deaths are not reported and disposal of the body is the responsibility of the next of kin, there is no mechanism by which a viscerotomy service on the South American plan can be made to operate.

In general, African tribes do not use common burial grounds for the village, although practice in this respect is highly variable. Burial in the compound or under the doorstep is common. At Isheri, a village a few miles north of Lagos on the Ogun River, there is marked diversity in this custom. Christians are buried in front of the house, Mohammedans are interred in the back yard, while pagans are put to rest under the living room floor. Burial in all regions is usually immediate, although the funeral may be held months later when it is more convenient for the mourners. These customs naturally increase the difficulties of learning of a death until it is too late for a specimen.

A colonial administration is quite different from that of the countries where viscerotomy has been successful. Especially in British colonies the aim has been to attain order with as little interference with the normal tribal life as possible. Regulations that apply directly to individuals have developed very slowly, the descent of administrative authority being through the chiefs to the subchiefs. Vital statistics are thus practically nonexistent and there is no information on births or deaths or on the population itself that has any validity save for tax purposes.

Somewhat exceptionally for Africa, the Belgian Congo has a system of viscerotomy which has been partially successful. In 193[illegible], after the results of the yellow fever immunity survey had become known, the collection of liver specimens was started; however, this service did not become really active until 1939. At the end of 1941 a total of 1,698 liver specimens had been reported, of which five were positive for yellow fever (Liegeois 1944).

Bugher believes that viscerotomy would probably have only limited value under the conditions prevailing in West Africa. He stated:

In South America viscerotomy has had its chief value in demonstrating where jungle yellow fever is active. The scattered and isolated cases picked up with such screening would be missed entirely if left to other diagnostic means. This is not so true in the case of epidemics with *A. aegypti* as the vector.

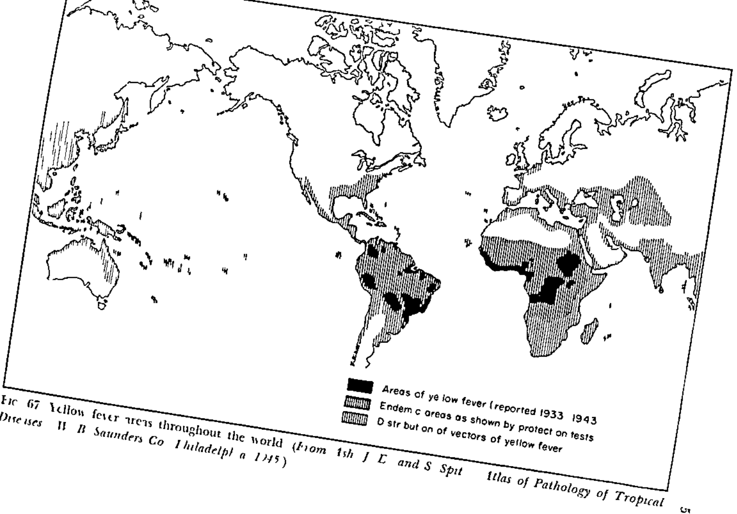
The picture in West Africa is quite different While back of the epidemic picture is the jungle infection the number of primarily forest acquired infections is apparently minute in comparison with those from *A aegypti* transmission I came to the conclusion that even if we had a viscerotomy service in West Africa that was more efficient than any in South America the chances of picking up jungle yellow fever in man would be very small and the possibility of recognizing such a case for what it was would be nil The entire picture would be so completely overwhelmed by the urban type that viscerotomy would be of no value in locating endemic foci of jungle yellow fever

Uganda has no legislation that would enable a viscerotomy service to exist nor did the officials believe such legislation was desirable A viscerotome was supplied to each medical officer in the outlying stations and a circular informing him of the instrument was sent out Following the threat of yellow fever aroused by the epidemic in the Nuba Mountains in 1940 a further supply of viscerotomes was obtained and distributed to medical officers in Kenya Tanganyika the Belgian Congo and the Sudan This measure failed to elicit liver specimens for examination in the Entebbe laboratory

At the time of the outbreak of yellow fever in the Bwamba forest region of Uganda in 1941 a European government official was trained in the use of the viscerotome He practiced viscerotomy himself and trained several Africans in the use of the instrument A moderate number of specimens was obtained Opposition to viscerotomy arose among the natives on religious grounds and the government put a stop to the practice of it

CONCLUSIONS

The widespread use of the mouse protection test and the systematic collection of liver specimens in many countries have resulted in a new concept of the importance of yellow fever as a cause of illness and death in the native populations of endemic regions Endemic yellow fever not only is a severe scourge among exposed rural populations but also constitutes a permanent reservoir of infection from which cities and towns may be reinfected Even with these intensive surveys the indubitable prevalence of yellow fever has been most difficult to determine In the absence of frank epidemics only routine viscerotomy can be expected to reveal the presence of the disease Most of the jungle yellow fever occurs in rural areas many of which are isolated and without medical services That this type of yellow fever can be



Fic 67 Yellow fever areas throughout the world (From Ash J E and S Spit Atlas of Pathology of Tropical Diseases W B Saunders Co Philadelphia 1945)

a devastating disease was evident in the 1931–1912 epidemics in southern Brazil which affected large areas and produced many thousands of cases. Another large epidemic of recent years occurred in the Nuba Mountains of the Anglo-Egyptian Sudan in 1940 (Kirk, 1941) with over 15,000 cases. In January 1950 a serious outbreak of yellow fever was observed in Bolivia in the Department of Chuquisaca in the Parapeti Valley and along the Río Azero. Some 850 cases with 230 deaths were reported. As far as the historical records go, yellow fever had not been known in that area since 1912. Because of isolation and poor transportation facilities outbreaks of this kind are difficult to combat.

Official reports of yellow fever obviously reflect only a small proportion of the cases that occur. It is thought, however, that a summary of these reports for recent years would be of interest. In Table 31 are given year by year and country by country the yellow fever deaths reported to the Office international d'Hygiene publique and the Pan American Sanitary Bureau from January 1927 to June 1950. In South America the diagnosis of yellow fever in fatal illnesses was generally confirmed by post mortem examination of liver tissue, but usually only a small proportion of the fatalities in a given outbreak were examined by autopsy or liver puncture. Many extensive epidemics of yellow fever in isolated areas have gone on completely unobserved by the health authorities. This is proved by subsequent immunity surveys.

In Africa, where viscerotomy is utilized to a very limited extent, the reported cases of yellow fever represent an even smaller proportion of those that have really occurred. The known list of yellow fever deaths does, however, give an impressive reminder of the widespread distribution of the disease.

As described elsewhere in this volume, a number of probable epidemic and endemic areas were selected both in Africa and in South America for intensive field studies on jungle yellow fever. Knowledge is still far from complete and future investigations may again require drastic modifications of present concepts.

It now appears that man is not an essential factor in the mechanism of transmission of jungle yellow fever. With this type of yellow fever he often takes no part in maintaining the virus; however, it has been observed on a number of occasions in South America that the human case is important as a possible mode of initiating aegypti-transmitted outbreaks.

Jungle yellow fever apparently depends on a supply of nonimmune sus

TABLE 31

YELLOW FEVER DEATHS

South and Central America

Country	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950
Bolivia						30														30				230
Brazil	4	83	443	5	24	34	14		8	179	165	248	118	151	12	6	1	6	37	1	2	3	5	
Colombia			23					3	10	101	50	15	8	25	31	18	23	9	17	6	68	19		2
British Guiana																			1			1		
Ecuador																							1	
Panama																						5	3	1
Peru											13				5					2	1	1	4	6
Venezuela															1			8	15	11		1		

Africa

Country	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950
Ivory Coast					25		3	29	9	1	5	1	11	2	1	7	6							
Dahomey						2			2	2	1	1												
French Guinea						25	9	1		1					3		2							
Niger					3		12	11	1	1			3											
Senegal					5	39	40	6			23	1				1	2							
French Sudan					24	48			1	3	1	2			1									
French Togo					6		9		3				1	1		1								
French Equatorial Africa											6	2	3	1	3					6				
Belgian Congo														1			2	2						
Gold Coast					15	2	13	2	4	3	51	15	2	1					4	1		2		2
Gambia								9																
Kenya																								
Nigeria					15		3		1	2	15		4							16	1			
Portuguese Guinea						12	2																	
Sierra Leone									2											1				
Spanish Guinea															4									
Sudan								1						6							1	1		
Upper Volta *					19	3																		
Mauritania					1																			

Source: Bernard 1934, Yellow fever deaths, Bull. Office intern. Hyg. pub. 1936–1946, Yellow fever deaths, Epidemiol. & Vit. Stat. Rep. 1948–1949, Yellow fever deaths, Pan American Sanitary Bureau Weekly Epidem. Reports 1949–1950.

* From 1933 this territory was divided between Ivory Coast, French Sudan, Niger.

ceptible animals in the forest and tends to die out rapidly in many places if that supply is depleted. In other outbreaks the virus persists much longer. The period between epizootics for given areas has been observed to vary from 3 to 10 years, probably depending on the time required to build up another large nonimmune population of susceptible animals and the accessibility of means for the reintroduction of virus. The evidence now indicates that the reservoir of jungle yellow fever tends to be maintained to a large extent in forested regions by monkeys and mosquitoes, both of which inhabit the upper strata of the forest much of the time. Infected monkeys therefore might pass through a district without producing human cases. In the jungle, just as in cities and towns, yellow fever virus would seem to lead a wandering existence, causing outbreaks whenever suitable conditions are found (Soper, 1947).

MODERN METHODS OF PROPHYLAXIS

Because endemic yellow fever is widely distributed both in South America and in Africa, and because it is maintained by inaccessible forest fauna, any attempt to eradicate the infection would seem foolhardy. In order to delineate the areas of endemic infections and obtain prompt reporting of epidemic outbreaks, however, it is important to continue systematic observations. With this information in hand, it will be possible to persevere in control measures in the endemic regions, not only for the protection of the local populations but to prevent spread of the infection to neighboring cities and countries. The speed of modern communications has multiplied the hazards of spreading infection, but fortunately the control methods available today are vastly superior to those of early yellow fever days. Effective techniques for *A. aegypti* reduction, as well as vaccines which afford full protection to human populations, have given health officers the necessary weapons for yellow fever control.

Obviously, the countries that face a constant threat in endemic yellow fever will be well advised to rid themselves of the urban vector. The successful campaigns of Brazil and Bolivia have demonstrated the feasibility of complete eradication of aegypti within the national borders.

Experience with the yellow fever vaccines now available has clearly shown that they afford full protection to individuals properly inoculated. Vaccination is at present the only method of protecting rural populations exposed to jungle yellow fever. Since it is the human infection that is usually a

principal factor in the spread of the virus from the jungle to towns or to other clean areas capable of being infected the vaccination of native populations in endemic regions may be expected to reduce both the incidence of cases in the immunized area and the likelihood of spread to other regions



Fig 68 Inoculation of yellow fever vaccine in Minas Gerais Brazil

A broad program of yellow fever control must take account of the following measures

1 A continuous investigation by viscerotomy immunity surveys and other methods to disclose the actual distribution of yellow fever
2 Anti aegypti measures
   a in endemic and potentially infectible areas eradication wherever feasible
   b in the cities ports and along main travel routes of endemic regions control when eradication is not feasible
   c in the principal cities and ports of regions that are nonendemic but threatened by invasion of the virus control
3 Vaccination
   a travelers to and from endemic areas
   b rural populations exposed to yellow fever
   c populations of exposed towns where anti aegypti control is not feasible

4 International regulations
  a anti-aegypti control of ports and international airdromes
  b disinsectization of aircraft
  c quarantine of potentially infected travelers

AEDES AEGYPTI CONTROL

The early campaigns for the control of mosquito-borne disease proved that it is not necessary to eradicate the vector species in order to stop the spread of infection. As the vector density is lowered by control measures a critical index (Carter 1931) is eventually reached below which transmission no longer occurs. Obviously this critical number to which the aegypti mosquitoes must be reduced to eliminate yellow fever decreases inversely with the proportion of susceptible people in a community.

In practice it was fairly easy to bring aegypti breeding down to the point where larval foci were limited to less than 5 per cent of the houses in the control area but attempts to reduce the house breeding index below 1 or 2 per cent were costly and difficult. Moreover any relaxation in the control measures usually resulted in an alarming increase of *A. aegypti*.

As long as yellow fever was thought to be an exclusively human disease transmitted by a single mosquito vector it was logical to attempt to rid the Western Hemisphere of the disease through temporary intensive campaigns to reduce aegypti density below the critical level in those cities known to harbor the disease. Under this plan no permanent mosquito control measures were undertaken since it was believed that once yellow fever disappeared from those cities it would also disappear from the surrounding areas. Because the cost of a continuous control organization is high funds for adequate services were generally available only during and for a limited period following known outbreaks of yellow fever.

The 1928–1929 epidemic of yellow fever in Rio de Janeiro forced a revision of the existing mosquito control methods and brought recognition of the danger of unchecked aegypti breeding in populated areas within striking distance of endemic foci. This situation in Brazil led to concerted efforts to develop more efficient methods of aegypti control and to reduce the costs to a level that would permit maintenance of control on a permanent basis. The results of these highly successful efforts have been fully described by those responsible for the Brazilian Yellow Fever Service during the critical

stages of the campaign (Soper and Wilson 1942 Soper Wilson et al 1943 Antunes 1948)

Following Brazilian practice the permanent campaign against *A aegypti* falls naturally into three phases

1 The initial clean up campaign for elimination of easily accessible foci This phase is similar in all respects to the early emergency yellow fever campaigns that rid localities of the disease but not of the vector
2 The discovery and elimination of the final hidden and inaccessible breeding places responsible for maintaining the species in spite of intensive anti aegypti measures
3 The maintenance of a permanent sentinel service to seek out and eliminate any reinfestation that may occur The final elimination of hidden breeding foci is achieved by (*a*) oiling or destruction of all water containers in which mosquito breeding is occurring (*b*) capturing adult mosquitoes to check on the persistence of aegypti and to locate hidden foci of breeding (*c*) searching out pupal producing foci in neighborhoods where aegypti mosquitoes persist in the face of routine control measures (Soper Wilson et al 1943)

In Brazil using the new methods of control the first zero indices in cities were obtained in 1932 After the attainment of a zero index it was often found that the persistence of viable eggs of aegypti or the reintroduction of aegypti from other localities not yet under control could cause a reinfestation of the cities Once the aegypti index has been brought to zero however it is possible to lengthen the cycle of house inspections from 1 week to 2 3 or 4 weeks and in many places to discontinue routine inspections completely Experience has shown that even a quarterly visit of adult capture squads is sufficient to indicate when and where services that have been discontinued should be reestablished

As the wide extent of jungle yellow fever in Brazil was discovered anti aegypti services were quickly expanded All towns and villages even the smallest in known infected areas and all towns of importance in previously infected or endemic areas were put under control Under this program ever larger areas were freed of aegypti and finally the possibility of eradicating this mosquito completely from Brazil appeared bright In 1942 eradication became the declared policy of the National Yellow Fever Service

The introduction of DDT into the anti aegypti campaign in 1946 added a powerful new tool to the available formidable armamentarium and gave

additional assurance of success It enabled the health officers to eliminate quickly the vector mosquitoes in exposed communities and thus render them nonreceptive to the invasion of virus DDT was used initially as a residual spray on the walls of houses but this was found to be expensive and unnecessary An emulsion of DDT sprayed on the household water containers outside and inside was sufficient The DDT thus applied had a long residual action and one treatment throughout a village was sometimes all that was required to eliminate aegypti breeding Excellent results were also obtained by adding DDT to the bilge water and to the water containers of boats on the Amazon River (Antunes 1948) By the end of 1949 *A aegypti* had been eradicated from 16 states five territories and the Federal District of Brazil A determined attempt is being made to eliminate the vector from the few remaining foci in the northeastern section of the country

In Bolivia according to Dr George Bevier representative of the International Health Division the Yellow Fever Service has maintained an anti aegypti campaign without interruption since 1932 Aegypti mosquitoes had a limited distribution in that country being most firmly established in the area around Santa Cruz de la Sierra Foci of infestation were also encountered along some of the principal rivers As a result of the control campaign *A aegypti* was eradicated from Bolivia by 1943 Small reinfestations that were easily controlled have since been discovered The last was cleared early in 1949 Thus Bolivia became the first country to eradicate *A aegypti* from its territory

To protect the countries trying to free themselves from the danger of reinfestation with *A aegypti*, the Directing Council of the Pan American Health Organization in 1947 approved plans to have the Pan American Sanitary Bureau coordinate the campaigns for the eradication of *A aegypti* from the Americas In accordance with this plan steps were taken to organize two regional campaigns one along the River Plate with the collaboration of the authorities of Paraguay Brazil Uruguay and Argentina and the other in the northern part of the continent with Venezuela Colombia Ecuador and the Guianas collaborating (Soper 1948 Pinto Severo 1948 Paoliello 1948) Reports showing the favorable progress of this eradication campaign appear at frequent intervals in publications of the Pan American Sanitary Bureau

Stimulated by the recent cases of yellow fever Panama is making a great effort to control aegypti By the middle of 1950 in only five towns in the re

public was evidence found of continued aegypti activity Complete eradication of the vector appears likely in the near future

Neghme (1950) reported an interesting experiment in the town of Iquique Chile by which the index of *A aegypti* was rapidly brought to zero by the introduction of DDT in a concentration of one part per million in the central water supply of the city The same procedure was repeated in the water supply of Antofagasta in January 1950 with equally satisfactory results Neghme believes that monthly or bimonthly applications of DDT to the water supplies may exterminate aegypti in these towns Experiments are under way to determine the optimum dosages and time intervals It must be pointed out that these towns are in a semidesert area in which almost all the water used by householders comes from the municipal system

In Africa the situation as regards *A aegypti* is different In addition to breeding around dwelling houses the species is widespread as a tree hole breeder and forest mosquito (Dunn 1927*b* Haddow 1945*a*) whereas in the Western Hemisphere it breeds almost exclusively in artificial containers near human habitations In Africa it is necessary to plan measures for the control of the mosquito to suit the local situation

Since the International Sanitary Convention for Aerial Navigation was signed in 1933 anti aegypti measures around international andromes have been emphasized

Prior to the Second World War the Office international d Hygiene publique issued periodic reports on the degree of infestation of African countries by mosquitoes capable of carrying yellow fever (Stanton 1937) These reports served to indicate the wide prevalence of *A aegypti* and to accentuate the vastness of the control problem

The Anglo Egyptian Sudan following the demonstration of yellow fever immunes in its territory by the protection test survey began effective aegypti control measures in many towns and cities as well as around airports and along communication lines Pridie (1936) reported Khartoum and Atbara free and Malakal almost free of aegypti A later account of this campaign was given by Lewis (1947) Aegypti are reported to have been eliminated from Port Sudan (Edwards 1949)

As a result of the Nuba Mountain outbreak of yellow fever in 1940 interest in the control of *A aegypti* in East Africa was greatly stimulated An endeavor was made to rid all ports all inland towns and all railway premises and airdromes in eastern Africa of that mosquito This campaign included all steamships and dhows plying on Lake Victoria and Lake Tan

ganyika and all railway premises (Yellow fever and East Africa 1941) Mass immunization in the rural and urban populations of the coast of Kenya was also undertaken During 1941–1942 over 320 000 persons were inoculated representing an estimated 90 per cent of the total population along 300 miles of the coast of Kenya (Kenya Medical Department Annual Report 1942)

Since the termination of the war few reports on aegypti control have been published from Africa The availability of DDT as an efficient and relatively inexpensive agent for the control of *A aegypti* should operate in the direction of encouraging African health officials to be less hesitant to undertake control measures when these are clearly indicated

Modern travel facilities have laid open to contamination all parts of the world in which conditions are favorable for the propagation of yellow fever virus It is now possible to circle the globe within the incubation period of the disease in man International sanitary regulations providing for mosquito control around ports and airdromes in critical areas disinsectization of aircraft and precautions against the transportation of infected persons from endemic to clean areas have become a necessity and to some extent have been adopted

A recent study made by Duguet (1949) on behalf of the World Health Organization summarized the present position in regard to the urgency of this problem and to the best available techniques for use in freeing air planes of insects Duguet concluded that disinsectization of aircraft when carried out by the proper means constitutes an effective prophylactic measure against the air transport of insect vectors of disease It cannot however by itself provide an absolute safeguard It is therefore imperative wherever environmental and climatic conditions are favorable to the virus to maintain and develop control measures against mosquitoes in the vicinity of air ports open to international air traffic in order to reduce the likelihood of mosquitoes entering aircraft making use of such airfields

CONTROL BY VACCINATION

Development of the 17D Vaccine Of the various methods of vaccination against yellow fever advocated prior to 1930 none gave satisfactory results The first effective method of immunization that could be adapted to human use was based on the work of Theiler (1930*a*) who reported a marked variation in the yellow fever virus produced by serial propagation in the brain

of the white mouse This mouse brain virus although possessing an enhanced neurotropism was found to have lost much of its viscerotropism and was therefore given consideration as the antigenic element in yellow fever vaccination Sawyer Kitchen and Lloyd (1932) used this mouse brain virus but also employed immune serum to give protection against its pathogenic activity This method proved efficient but cumbersome It was not adapted to mass vaccination because of the large amounts of immune serum required Sellards and Laigret (1932) vaccinated human beings with mouse brain virus without immune serum although other workers considered that the increased neurotropism of the virus rendered it potentially dangerous for human vaccination (*Findlay 1934b Theiler and Whitman 1935b*)

Shortly thereafter it was found that prolonged in vitro cultivation in embryonic tissue greatly reduced the viscerotropism of a highly virulent strain of yellow fever virus without causing a corresponding increase in its neurotropism (Lloyd Theiler and Ricci 1936) This cultured virus grown in mouse embryo tissue designated as the 17F strain was subsequently substituted for the mouse brain virus in the serum virus method of vaccination (Lloyd 1935 Soper and Smith 1938*a*) The reduction of viscerotropism in this cultured virus was not deemed sufficient to warrant its use without the protection of immune serum

Cultivation of yellow fever virus in vitro begun by Lloyd Theiler and Ricci (1936) involved the use of chick embryo tissue from which the central nervous system had been removed The virus known as 17D obtained by prolonged propagation in this medium has greatly diminished neurotropism and viscerotropism while still retaining its antigenic properties Subcutaneous inoculation of the 17D strain of virus into the rhesus monkey rarely produces a febrile reaction and the quantity of virus demonstrable in the monkey s blood is usually minimal Furthermore when injected intracerebrally into the rhesus monkey this virus does not produce fatal encephalitis It does still retain enough power to cause encephalitis in white mice The mortality from encephalitis in mice is not reduced but the incubation period is prolonged The rhesus monkey injected with 17D virus by any route is found to be completely immune to the subsequent inoculation of fully virulent strains (Theiler and Smith 1937*a*)

Theiler and Smith (1937*b*) after a prolonged and careful study in laboratory animals began human immunization with a single inoculation of this modified 17D strain of virus without immune serum The initial results with this method were so favorable that a field study of the new vaccine was

arranged in Brazil beginning early in 1937 (Fig. 69). As the titer of virus produced by multiplication in tissue culture is low, vaccine for human use is prepared from developing chick embryos inoculated with culture virus. The first lots of 17D vaccine for experimental use in Brazil were produced in New York, but by March 1937 vaccine production had been started in the yellow fever laboratories in Rio de Janeiro.

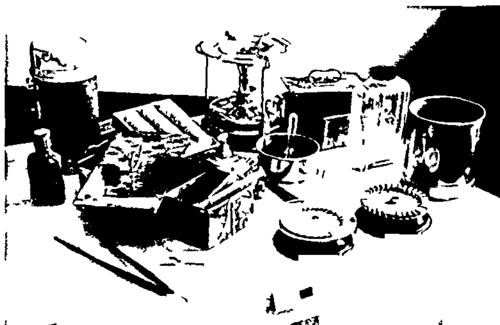


Fig. 69. Yellow fever vaccination equipment from a field kit. This kit is equipped with cultured 17D virus (1937).

Careful studies in which 17D vaccine was administered in the laboratory or in small groups in the field showed that between 97 and 100 per cent of those vaccinated had protective antibodies in their blood on subsequent test. Results of postvaccination tests indicated that approximately 95 per cent of those vaccinated under field conditions acquired immunity; usually protective antibodies could be demonstrated in the sera by the 14th day (Smith, Penna, and Paoliello, 1938).

Less than half of those studied gave evidence of virus circulating in the blood stream following vaccination. Virus was demonstrated in three cases on the 4th day, in three cases on the 5th day, in three cases on the 6th day, in two cases on the 7th day, and in one case on the 10th day. The quantities

virus in circulation were extremely small usually only one or two mice in the group receiving the test serum developed encephalitis

Reactions to 17D vaccine occurred in only 10 to 15 per cent of those vaccinated The symptoms most frequently noted were headache backache body pains weakness and malaise lasting from a few hours to a day or two These mild reactions usually came after an incubation period of 5 to 8 days Only 1 to 2 per cent of those vaccinated experienced reactions severe enough to cause loss of time from work In summary it can be said that the reactions to 17D virus are not severe enough to influence its general acceptance by the public Early restrictions on age have been removed because experience has shown that children of all ages can be safely immunized as can women in all stages of pregnancy

Very rarely perhaps once in a million inoculations according to published reports allergic reactions have set in following administration of 17D vaccine Sulzberger and Asher (1942) reported three cases of urticarial and multiform type skin eruptions that occurred in men of the United States Navy who had received injections of different lots of the vaccine Swartz (1943) published the record of a man who developed angioneurotic edema urticaria gastrointestinal symptoms and severe dyspnea a few minutes after receiving a single injection of cholera vaccine and one of yellow fever vaccine This man upon subsequent study was found to have a pronounced egg and fowl sensitivity Sprague and Barnard (1945) reported the case of a man who developed severe asthma within 15 minutes after receiving yellow ever vaccine he also showed edema of the face and generalized urticaria at he same time This patient gave a history of eczema and asthma He had ng been sensitive to white of egg

The evidence in monkeys is that an effective immunity protecting the ani l from challenge doses of virulent yellow fever virus develops as early as 5th or 6th day following inoculation with 17D vaccine Neutralizing body cannot usually be detected in the serum until a few days later tler and Smith 1937b Smithburn and Mahaffy 1945)

e efficacy of 17D vaccine in the prevention of yellow fever in human s has been clearly established In prevaccine days the incidence of tory infections in spite of the exercise of all known precautions was Berry and Kitchen 1931) Subsequent to the introduction of routine tion no cases of infection in the laboratory have been reported This ularly striking for not only was the number of individuals engaged w fever research greatly increased but also many precautionary

measures against exposure to virulent virus-containing materials in the laboratory were discontinued after the introduction of vaccination. In the field Soper and Smith (1938*b*) reported on the successful vaccination of almost 600 000 persons in Brazil, large numbers of whom lived among exposed populations during active outbreaks of jungle yellow fever. This experience added a mass of field observations to the already fairly conclusive laboratory experience. Local physicians and other observers reported a sudden reduction in observed cases in infected districts shortly after mass vaccination and cited instances in which individuals who failed to be vaccinated later contracted the disease in infected forests while inoculated members of the same labor gangs escaped the disease. Only eight cases of yellow fever were reported among those vaccinated. Onset of illness in two of these took place on the same day as vaccination, in four others between the 1st and 4th days following. It seems justifiable to conclude that these persons were infected with yellow fever before they were vaccinated. Bugher and Gast Galvis (1944) reported similar results from their experience with 17D vaccinations in Colombia. Of the 198 proved and 15 probable cases of yellow fever that occurred in Colombia after the beginning of 1937, the year in which large-scale vaccination was begun, all were among unvaccinated persons. Many of these cases originated in known endemic areas where over 90 per cent of the population was vaccinated. Cases continued to appear among the unvaccinated fraction in contact with the forest, although those inoculated and equally exposed were protected.

The important question of duration of immunity following 17D vaccination has been dealt with in several reports. Those giving results for the longest periods are summarized here. Smithburn and Mahaffy (1945) carried out mouse protection tests on the sera of 300 residents of Bwamba County, Uganda, 3 years after vaccination. They reported that over 90 per cent of the group had protective sera and that there was no evidence of decline in the incidence of immunity during the 3d year. Anderson and Gast Galvis (1947) tested 623 sera from persons in Colombia 5 years after vaccination. Ninety-three per cent of these individuals had protective antibodies in the blood. These authors concluded that revaccination up to 5 years is not necessary. Fox, Fonseca da Cunha, and Kossobudzki (1948) reported the results of 78 protection tests on sera collected in Brazil 6 years following immunization with 17D vaccine. While a high proportion of the sera showed protective antibodies, the authors believed there was some indication of a decline in the general level of immunity. By a more sensitive mouse protection test tech-

ique however all but four of the 78 sera showed protective antibodies Dick and Smithburn (1949) testing 183 sera from persons vaccinated with 7D vaccine in East Africa 6 years previously found that a very high per entage still showed immunity No significant difference in the immune esponse of children and adults was apparent The authors suggested that ealth regulations be amended accordingly and that yellow fever vaccina ion certificates be accepted as valid for at least 6 years after inoculation with otent vaccine

Difficulties encountered in the use of 17D virus include failure to im unize and the production of postvaccination encephalitis and jaundice The failure to immunize and the central nervous system reactions have been raced to further modifications in the 17D virus itself (Fox Kossobudzki nd Fonseca da Cunha 1943 Fox Lennette et al 1942)

A careful selection of substrains of virus 17D and the preparation of large eed lots of uniform material for the production of vaccine have eliminated he difficulties associated with properties of the virus itself Delayed jaundice ollowing vaccination is thought to be traceable to a contaminant virus ntroduced with the normal human serum that was used as a diluent of the hick embryo material in the preparation of the vaccine (Fox Manso et al 942 Sawyer Meyer et al 1944)

Because of reports that the serum base vaccine sometimes caused hepatitis fforts were made to modify the technique for the preparation of vaccine o that the virus containing chick embryo material could be used without he addition of human serum The Yellow Fever Laboratory in Brazil began n December 1940 to eliminate serum completely in the preparation of 17D accine using instead the infected chick embryo extract alone The serum ase vaccine however continued to be generally employed while studies on he aqueous base product were under way In February 1941 the Rocky Mountain Laboratory of the United States Public Health Service at Hamil on Montana began to make experimental lots of 17D aqueous base vaccine A field study of the comparative behavior of serum base and aqueous base accines in Peru showed them to be equally efficacious (Hargett Burruss and Donovan 1943)

The Yellow Fever Laboratory of the International Health Division in New York also started experimentation with the preparation of aqueous ase 17D vaccine in 1941 Although under laboratory conditions the vaccine appeared to be satisfactory the relative lack of field experience with it made a change from serum base vaccine appear unwise at that time However

after the report of an outbreak of acute hepatitis in March 1942 among United States Army personnel given the serum base vaccine the change was made Since that time only aqueous base vaccines have been produced (Sawyer Meyer et al 1944) The aqueous base product was also put into production by the United States Public Health Service and released for general use

Technique of 17D Vaccine Production By the present technique of vaccine production fresh fertile hen eggs are incubated for 7 to 9 days Eggs that show a viable embryo are then inoculated with 0 05 cc of 200th to 300th subculture passage material of 17D virus via a small hole drilled through the shell After the inoculation holes have been sealed with phenolized sterile paraffin the eggs are returned to incubate at 99°F for 4 days to permit the multiplication of virus

Before harvesting the eggs are candled with great care to eliminate those in which the embryo appears dead or abnormal A disinfectant is then applied and a ring is burned around the shell of each egg with a sharply pointed intensely hot flame (Penna 1939 Pickels 1942) The shell top is turned back and the embryo removed with sterile forceps The head is severed from each embryo just back of the eyes and discarded

The next step in the procedure is the maceration of the embryonic tissue into a fine pulp A colloidal mill is used for this purpose A beaker of embryos without heads is introduced through a funnel into the mill and sterile distilled water is added in the ratio of 25 cc for each 100 cc of embryo volume The milled pulp is collected in two liter wide mouthed bottles A sample from each bottle is removed for sterility tests and virus determination and the bottle is then sealed airtight with a sterile rubber stopper The pulp is shell frozen and stored in a CO cabinet at a temperature of approximately −75°C

Frozen pulp that proves to be sterile and to have an adequate virus content is thawed in a water bath at 37°C The pulp is then transferred to 250 cc centrifuge bottles and centrifuged for 40 minutes at 2 000 r p m The supernatant fluid is then passed through a column of sterile brass wire sieves to remove fat and other insoluble material The clarified embryo juice with its small content of water added during milling is diluted with an equal volume of sterile distilled water The juice could be used as vaccine without this additional dilution with water

The vaccine is then packed in ampules and rapidly shell frozen and desiccated in the frozen state The desiccation is accomplished by cold surface

condensation using apparatus similar to that described by Bauer and Pickels (1940) Eighteen to 20 hours are necessary to desiccate the usual lot of vaccine After desiccation is complete dry nitrogen is introduced into the drying apparatus and the ampules are sealed off with an oxygen flame

The term vaccine lot refers to material that is processed together as a unit and is of uniform composition

With each ampule of vaccine a bottle of sterile diluent for the rehydration and dilution of the vaccine is supplied The diluent consists of 0 2 per cent of sodium chloride in distilled water The diluent is put up in bottles of two sizes The smaller bottle contains 11 cc and is designed for the dilution of the vaccine from the ampule containing the dried equivalent of 1 cc of fluid vaccine Each ampule should yield 20 doses The larger bottle contains 55 cc of diluent to be added to 5 cc of dried vaccine It yields 100 immunizing doses (Hargett Burruss and Donovan 1943 Sawyer Meyer et al 1944)

Manufacturing Standards In 1945 the Standing Technical Committee on Health of the United Nations Relief and Rehabilitation Administration adopted standards for the manufacture and control of yellow fever vaccine (Standards for the manufacture and control of yellow fever vaccine Epidem Inf Bull 1945) These standards were developed on the basis of 8 years of experience with the use of 17D vaccine and apply all known safeguards to protect the public from untoward reactions and to insure a satisfactory product The principal points set forth in these standards are

1 The dried vaccine shall contain not more than 1 per cent of moisture (preferably not more than 0 5 per cent) as determined by the phosphorus pentoxide vacuum method

2 Each lot of seed virus must be tested by intracerebral inoculation in not less than six rhesus monkeys that have been proved susceptible to yellow fever These monkeys must be observed for 30 days for symptoms of encephalitis and tested for circulating virus and for postinoculation immunity to yellow fever Only seed virus that affords the monkeys satisfactory safety against encephalitis as well as immunity to yellow fever is acceptable for the preparation of vaccine

3 The finished dried chick embryo pulp when rehydrated to its original volume shall contain not less than 150 000 M L D for mice of 17D virus per cc of vaccine at the time of passing final potency test

4 A safety test in the guinea pig shall be made on each lot of finished vac

cine Four to 5 cc shall be injected intraperitoneally into each of two or more normal guinea pigs The animals shall be observed for 7 or more days during which time no significant clinical manifestations shall occur

5 Sterility of the product must be maintained at all times while processing The finished vaccine shall be sterile as indicated by tests on ampules selected at random Duplicate cultures shall be made with incubation at 37 and 22°C

6 The ampules of vaccine shall be properly labeled to include the date of manufacture the date of issue and the expiration date Between the date of issue and the expiration date which may not be an interval of more than one year the product must be kept constantly at a temperature below 5°C (41°F)

7 Vaccine shall be shipped in a suitable container adequately packed in carbon dioxide ice

Mass Immunization Programs No accurate record of the total number of persons inoculated with 17D vaccine exists but some indication of the magnitude of that number may be gained from the fact that the Yellow Fever Laboratory of the International Health Division alone distributed more than 28 000 000 doses of 17D vaccine Of this amount approximately *18 000 000 doses went to the United States* Armed Forces during the war years Over 6 000 000 doses were sent to Africa for distribution to both civil and military medical services from the yellow fever laboratories located at Lagos Entebbe and Johannesburg The remainder went to India South America and in small amounts to miscellaneous agencies that had legitimate need for yellow fever immunization

In South America most of the vaccine administered was produced by the yellow fever laboratories of Rio de Janeiro and Bogotá While figures for the numbers inoculated are not complete the following reports are of interest

Country	*Period*	*Number vaccinated*
Brazil	1937 1949	5 713 067
Colombia	1937 March 1950	1 527 268
Bolivia	1938 June 1950	211 163
Peru	1937 May 1950	186 3[illegible]0
Venezuela	1938 June 1950	361 662
Total		7 999 530

During this period a number of other laboratories were producing and applying 17D vaccine. Among these were the United States Public Health Service, Hamilton, Montana; the Wellcome Research Institution, London; the Pasteur Institute, Paris; and the South African Institute for Medical Research, Johannesburg.

Through the kindness of Brigadier G. S. K. Boyd, director of the Wellcome Laboratories of Tropical Medicine, the following information was obtained. In London approximately 125 000 individuals were inoculated against yellow fever with 17D vaccine between 1937 and 1950. During this period about 1 000 000 doses of 17D vaccine were distributed by the Wellcome Research Institution to other centers in Great Britain. It is not known how many inoculations were actually carried out with this vaccine, but probably at least half a million persons, mostly in the military forces, were vaccinated. Stefanopoulo and Duvolon (1947) reported satisfactory results with 17D vaccine in the immunization of 20 000 individuals at the Pasteur Institute in Paris in the period 1936–1946. In this group were 2 470 children aged 15 days to 10 years. No untoward reactions were noted among them.

Under urban conditions or in regions where communications are reasonably good there is little difficulty in the application of 17D vaccine to large populations. In primitive areas the transportation of the necessary refrigerating apparatus and the equipment necessary for the proper application of the vaccine offers real difficulties, and a simpler method of immunization is desirable. Also, the cost factor in the immunization of large native populations is an important consideration, particularly as it now appears that immunizations on a mass scale must be continued indefinitely. The cost of producing 17D vaccine has been estimated at about US$0.022 per dose in New York, and at about US$0.025 per dose in Rio de Janeiro (Soper and Smith, 1938*b*). This figure is low, but when the cost of application is added, mass immunization by this method becomes a burdensome expense. Attention has been given, therefore, to the development of a safe, reliable, but cheaper method of applying 17D vaccine. In this search the experience of the French yellow fever specialists in West Africa has furnished an excellent lead.

Scratch Methods of Vaccination. Vaccination against yellow fever has been practiced in the French territories of western Africa since 1934. The French vaccine was developed from the French strain of virus modified by mouse brain passage. The first method employed (Laigret, 1934) consisted of three injections of mouse brain virus suspended in glycerin at room tempera-

ture and mixed with phosphate powder Later Nicolle and Laigret (1935) initiated a single dose method of vaccination employing mouse brain virus coated with a layer of egg yolk or olive oil or with a double layer of both agents The object of this method was to retard diffusion of the virus from the site of inoculation

By the end of 1935 over 23 000 persons in French West Africa had received this Laigret vaccine Sorel (1936) and Mathis Durieux and Mathis (1936) studied the incidence of severe reactions to this vaccine and concluded that a significant number of prolonged reactions involving the nervous system were to be expected

Convinced of the necessity for the immunization of the native masses against yellow fever the Pasteur Institute of Dakar sought a simpler method which could more easily be given wide application Its primary object was to replace subcutaneous inoculation which requires a considerable number of syringes and needles rigorously sterilized with immunization by the simple application of virus to cutaneous scarifications

Peltier Durieux et al (1940) reported on a new method of immunization with the French neurotropic strain of virus Using material from the 238th passage of the virus through mouse brains six human volunteers four white and two Negro were vaccinated by applying the virus to scarified areas on the skin In five of them virus appeared in the blood and all developed antibodies to yellow fever The authors then tested a mixture of neurotropic yellow fever virus and smallpox vaccine to determine whether vaccination against both diseases could be performed simultaneously This scratch method of vaccination was tried on 741 human subjects without accident

A vaccination campaign to protect 100 000 inhabitants of Senegal was promptly authorized and carried out during the months of May June and July 1939 Those vaccinations were performed in a region where no case of yellow fever had been noticed for a long time to facilitate observation of any accidents that might occur among both the vaccinated individuals and their neighbors The vaccines were very well tolerated The neighboring population was very carefully observed and no manifestation of yellow fever not even the slightest was noticed despite the prevalent breeding of *A aegypti* (Peltier 1947)

In view of the numerous dangers confronting the troops stationed in French West Africa the public authorities decided in the latter part of 1941 to make vaccination against yellow fever by the method developed at the Pasteur Institute of Dakar compulsory for all the military and civilian

populations of those regions It was further decided that the yellow fever vaccination should be associated with the vaccination against smallpox whenever the administration of the latter was deemed necessary

Peltier (1946) stated that the vaccine is made from the brains of mice inoculated with the French strain of virus of the 256th to the 258th passage in mice The mouse brains are dried in a vacuum chamber containing calcium chloride at −25°C The dried brains are finely ground in a mortar with infusorial earth to which powdered kaolin is added To a measured volume of brain powder two volumes of sterile inert powder are added The mixture is again dehydrated under vacuum at −25°C for 24 hours The powder is then distributed into ampules each containing $^1/_{10}$ of a brain which represents 100 doses of vaccine The ampules are sealed under vacuum and stored at −4°C The vaccine retains its potency for 2 months provided it is kept under refrigeration It may be transported at ordinary temperatures if such transportation does not take more than a few days For use the powder is placed in a mortar and 2 cc of sterile neutral gum arabic solution is added and mixed with the powder Dried smallpox vaccine is frequently mixed with the yellow fever vaccine The mixing is done in the mortar before adding gum solution Two drops of the suspension are put on the skin in the deltoid region and through each drop two parallel scarifications 0 5 cm in length are made The gum dries and provides a covering The vaccinations should be carried out during the least hot seasons preferably in the early hours of the day and always in the shade The vaccinated groups should be kept under observation for 5 minutes to make sure the vaccine is not wiped off

Peltier (1947) reviewed the experience with the method during the 4 year period 1942–1946 The total of simple and mixed vaccinations against yellow fever had then reached 11 330 735 for a total population of about 16 millions in French West Africa In 106 sera from a village vaccinated 4 years previously 85 9 per cent gave positive protection test results From a village vaccinated 7 years previously 82 per cent of 72 sera gave positive mouse tests

At the Fourth International Congresses on Tropical Medicine and Malaria in 1948 Peltier gave a progress report on yellow fever vaccination in French West Africa By that time just over 20 000 000 vaccinations had been done 2 9 million with yellow fever only and over 17 million with the combined vaccine Many individuals have been vaccinated repeatedly since the campaign began Ten to 15 per cent experienced reactions from the 5th

to 7th day after vaccination These reactions are considered a response to the invasion of the blood by the virus A small number of persons experienced delayed reactions from the 12th to the 15th day which indicate invasion of the nervous system by the virus These late reactions usually clear up after 5 to 6 days Grave reactions have been very rare To determine the duration of immunity a total of 3 197 postvaccination sera was tested at intervals of 1 month to 7 years after vaccination The percentage protected varied from 87 to 95 per cent during the first 5 years but appeared to decline a little thereafter The present plan of the health authorities is to attempt to revaccinate the native population every 4 years

An interesting comparison between the scarification method of yellow fever vaccination using mouse brain virus and the subcutaneous inoculation of 17D vaccine was conducted in France in 1945 under the auspices of the Quarantine Commission of the Expert Committee on Health of the United Nations Relief and Rehabilitation Administration Six hundred and thirty young adult French soldiers none of whom had been out of France were divided into three groups of 210 each Group A received French neurotropic vaccine by the scratch method Group B the same vaccine mixed with small pox vaccine also by scarification and Group C the 17D vaccine subcutaneously (Dakar Yellow Fever Vaccine 1946)

There were no severe reactions to the vaccination in any of the three groups Sixty nine individuals in Group A 23 in Group B and 19 in Group C experienced some reaction around the 6th to 7th day The most common *symptoms complained of were headache stiffness and ocular and periorbital* pains

The men were bled 36 to 37 days after vaccination One part of each serum specimen was held in reserve and the other two were sent for antibody tests to Dakar and to the United States Public Health Service in Montana On 92 sera there were discrepancies between mouse protection test results at the Montana and Dakar laboratories There was sufficient reserve from 86 of these sera for a further test in the laboratory of the Yellow Fever Service in Rio de Janeiro

In Group A 96 88 per cent of the men had developed neutralizing antibodies as tested in Dakar and 100 per cent as tested in Montana Group B had 97 47 per cent positive when tested at Dakar and 98 96 per cent positive in the United States There was thus no doubt about the immunizing potency of the mouse brain vaccine applied by scarification either alone or in combination with smallpox vaccine

In Group C the percentage of positive sera as tested in the three laboratories was 61.29 per cent. Another 9.18 per cent of the sera gave inconclusive results while 26.53 per cent of the sera failed to protect mice. It appears from this field trial that the Dakar vaccine produces a greater degree of immunity as measured by serum antibody response. This is accompanied by a greater danger of serious neurologic reactions from the neurotropic vaccine as well as the risk of extraneous infections from latent viruses of mice that may be pathogenic for man. It appears reasonable therefore in view of the satisfactory experience with 17D vaccine in large scale immunization campaigns over a period of 13 years that its greater safety would recommend it above the Dakar vaccine for general use. In making this recommendation one would wish to acknowledge the splendid accomplishment of Peltier and his colleagues in developing an efficacious but simple method for the mass vaccination of the millions who urgently required protection in French West Africa. That campaign has been highly successful and is certainly one of the most ambitious public health measures ever put into effect.

After a study of the Dakar methods of producing and applying neurotropic yellow fever vaccine an attempt to develop a compatible scratch vaccine with 17D virus was started in 1947 by the staff of the Yellow Fever Research Institute at Lagos Nigeria. Chick embryos infected with 17D virus were ground together with gum arabic solution and the homogenized mixture then dried to powder form. When mixed with water this vaccine is easily applied by the scratch technique. Trials on monkeys and small groups of humans gave successful results. Tests on larger groups are contemplated and experimentation on the technical problems involved in the preservation of the vaccine and in the combination of the new vaccine with smallpox vaccine is under way. It would appear likely that the scratch method of vaccination on account of the low cost of production and application may prove to be the method of choice for the immunization of large rural populations. The subcutaneous inoculation of 17D vaccine still appears to be the preferable means of immunizing individuals or small urban groups.

INTERNATIONAL CONTROL MEASURES

The first international measures to prevent the spread of disease were probably the maritime quarantines instituted during the pandemic of bubonic plague that devastated Europe in the fourteenth century (Howard Jones 1950). Through the years quarantines were also applied against

cholera but the complete lack of agreement as to the mode by which epidemic diseases were spread created a corresponding lack of agreement on means of preventing them There was no consistency in the quarantine regulations of *different nations or even of different ports of the same nation* These circumstances led to a series of international sanitary conferences the first of which was held in Paris in 1851 An international convention was drawn up but only three nations ratified it A second international conference consisting only of diplomats was convened in Paris in 1859 but produced no results During the remainder of the nineteenth century eight other international sanitary conferences were held all in Europe with the exception of one held in Constantinople These conferences were of an essentially diplomatic character and accomplished little of value

The first International Sanitary Convention was signed as recently as 1892 This convention which was unanimously ratified by the 11 signatory powers was directed primarily toward protection against the introduction of cholera via the Suez Canal which had been opened in 1869

In 1903 on the initiative of the Italian government the 11th International Sanitary Conference was convened in Paris The result of this meeting was the International Sanitary Convention of 1903 which unified the earlier conventions of 1892 1893 1894 and 1897 in the light of contemporary scientific knowledge This convention ratified by most of the participating states was the first to introduce some measure of international uniformity against the importation of cholera and plague It was superseded by the conventions relating to maritime traffic of 1912 and 1926 the latter was modified in 1938 and again in 1944 The *International Sanitary Convention for Aerial Navigation* was signed at The Hague in 1933 and this was amended by a new convention in 1944

The present International Sanitary Conventions are imperfect instruments for the control of epidemic disease especially as their signatories may delay ratification for years

Late in 1949 the Expert Committee on International Epidemiology and Quarantine of the World Health Organization drafted a set of International Sanitary Regulations to replace the present conventions According to information received from Dr G Stuart Chief Sanitary Conventions and Quarantine Section World Health Organization these have been distributed to governments and to appropriate international bodies for study and comment and will probably be taken up for consideration by the World

Health Assembly in 1951 If agreed upon then the regula into effect in 1952 Future alterations that may be needed national quarantine practice to new scientific discoveries by decision of the assembly

It is expected that within a few years the regulations will b by a series of technical annexes outlining the methods by wh visions may be most efficiently implemented

The existing sanitary conventions are administered by th ventions and Quarantine Section of the World Health Org tariat and it is expected that the new sanitary regulations into force will likewise be administered by that section T rently receives and issues notifications of outbreaks of epidem whenever possible it settles differences arising between ing quarantine measures and requirements The few disput be settled in that way are submitted to the Expert Committ

An important principle recently affirmed by the Second Assembly to govern future international sanitary legislation should not rely entirely on measures taken at their fronti entry of epidemic diseases but that they should endeavor selves non receptive to such diseases by internal action against the insect vectors of disease or towards mass immu population (Biraud 1950)

One result of the many sanitary conferences has been an nition of the need for an international health agency The Sanitary Bureau was established in 1902 as a regional health cipal function during the first 20 years of existence was th dissemination of information regarding the distribution c eases in American seaports It was not until 1908 that the Offi d'Hygiène publique came into being The main work of tl has been the administration and revision of the Internation ventions The 1912 convention which included provisions plague and yellow fever was signed by 41 member states b into effect until after the First World War The convention

come members of the League. This was the main reason for the continuation of the Office international d'Hygiène publique after the establishment of the Health Organization of the League of Nations in 1923. Fortunately the two agencies were able to collaborate closely.

Up to 1925, countries attempted to keep informed of epidemic diseases abroad through their consular agents in foreign ports or through exchange of information by diplomatic channels. This system proved to be too slow and too inaccurate to check international spread of disease. When the Health Organization of the League of Nations set up an epidemiologic station in Singapore in 1925, some 180 ports in the East volunteered to keep this station informed by telegram each week of their sanitary condition. The 1926 sanitary convention formally entrusted the Office international d'Hygiène publique with the responsibility for the collection and distribution of epidemiologic information. The same convention took into account the need for the distribution of epidemiologic intelligence on a regional basis and formally recognized the Pan American Sanitary Bureau and the Singapore bureau for that purpose. The efficiency of the services sponsored by the Office international d'Hygiène publique and the League of Nations gradually improved until the onset of war in 1939 forced the international exchange of such information to a low ebb. Under the United Nations Relief and Rehabilitation Administration and the World Health Organization services have been resumed and are again being put on a satisfactory basis.

The main provisions for yellow fever control measures in the International Sanitary Convention of 1926 (as amended in 1944) and in the Sanitary Convention for Aerial Navigation of 1933 (as amended in 1944) are:

1. Each government shall take all possible measures to establish the existence or nonexistence of yellow fever within its territories.
2. Each government shall immediately notify the other governments, and at the same time the World Health Organization, of the first recognized case of yellow fever. Every notification shall be accompanied by detailed information as to the locality where the disease has occurred, the date of its appearance, its source, and its type, the number of established cases and the number of deaths, the extent of the area or areas affected, the presence and relative abundance (index) of *A. aegypti*, the control measures taken. Subsequent weekly bulletins to keep the governments informed of the progress of the epidemic are required.

3 Governments shall be obliged to take measures to prevent the embarkation of persons sick with yellow fever and to prevent mosquitoes from gaining access to ships or aircraft

4 Regulations (set forth at length) shall be observed for handling ships carrying cases of yellow fever abroad for ships suspected of being infected and for ships regarded as healthy but coming from a yellow fever infected port

5 Governments shall render and maintain free from *A aegypti* (*a*) ports and their surroundings in endemic areas and (*b*) ports not situated in endemic areas but exposed to the risk of the introduction of the disease

6 Regulations are set forth imposing a responsibility for each country to make sanitary all airdromes that are located in a region in which yellow fever exists Insect vectors of yellow fever must be eliminated at (*a*) airdromes and their surroundings in endemic yellow fever areas and (*b*) airdromes not situated in endemic areas but exposed to the risk of the introduction of the disease Disinsectization of aircraft shall be carried out at each airdrome within an endemic yellow fever area particularly on departure from the last airdrome in an endemic yellow fever area Upon arrival at the first airdrome of call aircraft that have proceeded from endemic yellow fever areas shall be disinsected

7 Governments shall use their best endeavors to secure that all persons who are likely to land in an endemic yellow fever area shall be inoculated against yellow fever 10 days before arrival in the area that inoculation against yellow fever shall be required for all regular staff employees and crews using authorized airdromes situated in endemic yellow fever areas that any person not in possession of a valid anti yellow fever inoculation certificate shall be considered to have been exposed to the risk of contracting yellow fever during the period of his stay in an endemic yellow fever area

8 All persons traveling from an endemic yellow fever area to one in which yellow fever does not exist but in which there may be conditions that permit its development shall be dealt with at the first stopping place in the latter area as follows (*a*) if they are in possession of a valid anti yellow fever vaccination certificate they shall be allowed to proceed without any quarantine restrictions in respect to yellow fever (*b*) if they are not in possession of a valid vaccination certificate they may be isolated in properly screened quarters until the certificate becomes valid or until 6 days have elapsed

In order to give meaning to the provisions of the sanitary conventions it was necessary to define the existing endemic areas of yellow fever in the world Under the terms of the International Sanitary Convention for Aerial Navigation 1944 this obligation was imposed on the United Nations Relief and Rehabilitation Administration in consultation with the governments concerned and within the Western Hemisphere with the Pan American Sanitary Bureau This function of the United Nations Relief and Rehabilitation Administration has now been assumed by the World Health Organization

During its session of December 1–6 1949 the World Health Organization Yellow Fever Panel recommended the delineation for purposes of quarantine control of certain endemic areas in Africa and America The delineation so recommended was endorsed by the Executive Board during its session January 16–February 2 1950 and accepted by the Third World Health Assembly on May 20 1950

In Africa the World Health Organization Yellow Fever Panel (United Nations WHO 1950) gave the following delineations

> From the mouth of the River Senegal along that river eastwards to the 15°N parallel of latitude thence eastwards along that parallel to the eastern border of the Anglo Egyptian Sudan thence northwards along the north western boundary of Eritrea to the Red Sea Coast thence southwards along the eastern coast of Africa to the northern boundary of the French Somali Coast thence along that boundary successively westwards southwards and eastwards to the eastern coast of Africa and thence along this coast to the southern boundary of the Protectorate of Kenya thence westwards along that boundary and the southern boundary of Kenya Colony to its junction with the southern border of the Uganda Protectorate and thence along this and the eastern border of Ruanda Urundi and of the Belgian Congo to the 10°S parallel of latitude thence westwards along that parallel to the west coast of Africa thence northwards along the west coast of Africa to the mouth of the River Senegal including the islands of the Gulf of Guinea The whole territory of Nyasaland Protectorate the Barotse Province and the Balovale District in the Western Province of Northern Rhodesia together with the territory to the South of Barotseland lying between the 23° and 25°E meridians of longitude down to the 21°S parallel of latitude are also included in the endemic area The port of Massawa in Eritrea and an area 10 kilometres in radius from the centre of the town of Asmara in Eritrea as well as the territory of the French Somali Coast including the port of Jibuti are excluded from the endemic area The continued exclusion of

these areas is however contingent on their maintenance of an *Aedes aegypti* index not exceeding 1% in the port of Massawa, in and around Asmara and in the port of Jibuti as reported quarterly to WHO (Endemic Yellow Fever Areas Weekly Epidemiol Rec 1950) (Fig 70)

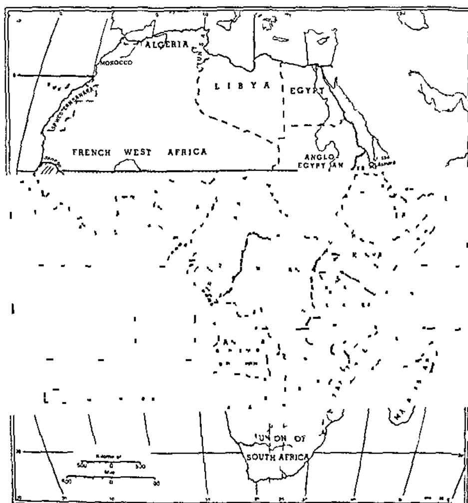


Fig 70 Endemic yellow fever area in Africa as recommended by the World Health Organization Yellow Fever Panel in December 1949

The American endemic yellow fever area is defined as follows

This area is bounded by a line beginning on the Pacific Coast of Colombia at the 5°N parallel of latitude and extending east along that parallel of

latitude to the eastern slopes of the Central Cordillera to an elevation of 2 000 metres thence southwards along the eastern slopes to the Central

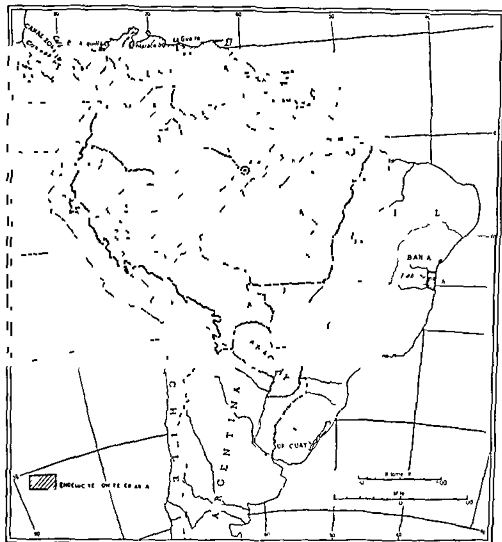


Fig 71 Endemic yellow fever area in South America as recommended by the World Health Organization Yellow Fever Panel in December 1949

Cordillera and the Andes Mountains at the same elevation to the boundary of Bolivia and Argentina thence eastwards and northwards along the southern and eastern boundaries of Bolivia to the 15°S parallel of latitude thence eastwards along that parallel of latitude to the western boundary of the State

of Goiaz, thence northwards along that boundary and the western boundary of the State of Maranhao to the Atlantic Coast, thence along the Atlantic and Caribbean coasts of America to the eastern boundary of Costa Rica, thence along that boundary to the Pacific Coast and thence along the Pacific Coast of Panama and Colombia to the 5°N parallel of latitude. In addition the Ilheus and Itabuna Districts in the State of Bahia in Brazil bounded on the north by the River Contas, on the west by the 40°W meridian of longitude, on the south by the River Pardo and on the east by the Atlantic Ocean are included in the endemic yellow fever area. The ports of Belem and Manaos in Brazil, Cayenne in French Guiana, Paramaribo in Surinam, Georgetown in British Guiana, the Caribbean ports of Venezuela and Colombia, the cities of Caracas in Venezuela and Bogota in Colombia, together with the ports of the Republic of Panama and the Panama Canal Zone are excluded from the endemic yellow fever area. The continued exclusion of these ports and cities is, however, contingent on their maintenance of an *Aedes aegypti* index not exceeding 1%, as reported quarterly to WHO (Endemic Yellow Fever Areas, Weekly Epidemiol. Rec., 1950) (Fig. 71).

As regards America, the delineation of endemic areas is based on reports of clinical cases, on the results obtained from routine viscerotomy, on immunity surveys of human and animal sera, and on the reporting of low *A. aegypti* indices.

In Africa the limits of the endemic area are based largely on the results of immunity surveys.

The Yellow Fever Panel continued as follows:

In the light of many years epidemiological observations in South America, it is known that, in regions in which *Aedes aegypti* is *not* present, jungle yellow fever may prevail among forest animals and cause accidental human infections without the production of outbreaks based on man-to-man transmission.

On the basis of the non-human reservoir, of the vector species and of the persistence of the infection, it is possible to distinguish between:

enzootic yellow fever areas, free of *Aedes aegypti*, and in which the virus is present and persists among animals over long periods of time, with the production of accidental human infections (woodcutters, hunters, etc.);

epizootic yellow fever areas, free of *Aedes aegypti* and in which the disease occurs periodically among animals for short periods of time;

endemic yellow fever areas, with *Aedes aegypti*, and in which the virus is present and persists among animals over long periods of time;

epidemic yellow fever areas in which cases caused by transmission of the virus by *Aedes aegypti* are found

As appears from the above definitions the difference between the endemic and epidemic area is that transmission from man to man by *Aedes aegypti* is potential in one and observed in the other

Since there may be failure to observe transmission by *Aedes aegypti* for considerable periods of time measures to be applied against arrivals from endemic and epidemic areas will be identical

Measures may be applied permanently against arrivals from endemic areas but the application of measures in epidemic areas may be restricted to one year after the last diagnosed human case or to two months after the reduction of the *Aedes aegypti* index to 1% or less

For the purpose of international quarantine the African endemic area is as delimited above It is to be expected that long term epidemiological observations will eventually permit the breaking down of the African yellow fever zone into areas as clearly defined as in America thus making it possible to adapt quarantine measures to the true degree of risk

The experts [of the World Health Organization Yellow Fever Panel] unanimously agreed [at their December 1949 meeting] that for purposes of quarantine the certificates of inoculation against yellow fever should be valid as from the 10th day to the end of 6 years following inoculation They stressed however the fact that there was considerable evidence to show that effective immunity was established as early as the 7th day following inoculation and persisted for a number of years beyond the six recommended (United Nations WHO 1950)

10 COSTS AND MAN POWER

by GEORGE K. STRODE, M.D.

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THIS BOOK has presented the story of one of the large undertakings of The Rockefeller Foundation for the control of a single disease. Little reference has been made to the character of the staff or to the cost of the enterprise, yet it is obvious that the achievements depended in large measure on generous technical and material support. It is therefore fitting to record as completely as possible what the facts were. The data presented relate particularly to The Rockefeller Foundation for the very good reason that its records are available for consultation, while those of the cooperating governments are but partially so.

When carried out on a global scale, the investigation and control of a disease of world importance, such as yellow fever, enjoy an enhanced likelihood of success if three essential elements are assured. The first requirement is cooperation of those sovereign states in which the disease is prevalent or is considered by them to be a potential menace to national security and development. Secondly, there must be provision for personnel, adequate in numbers and appropriately diversified in technical competence, to assure a suitable organization and administration of the epidemiologic investigations and control operations in field and laboratory, irrespective of geographic location. And finally, the financial resources must be sufficient to meet requirements for effective operation. The time element can rarely be predicted with accuracy and usually exceeds preliminary estimates. When the Foundation launched its program for the eradication of yellow fever from the world in 1916 it felt confident that the task could be completed in a relatively brief period, but by 1933 it was clear that an early denouement was out of the question, so that disappointment in that respect was inevitable. The Foundation decided, nevertheless, to stick to the task, and in return was rewarded by an array of knowledge and understanding of yellow fever that proved sufficiently ample to secure adequate control. The disease has ceased, as was its wont, to excite terror and panic in the world.

The 11 countries with which The Rockefeller Foundation cooperated technically as well as financially were spread over three continents. In Africa they were Nigeria and Uganda. In North and Central America they included Cuba, Guatemala, El Salvador, Mexico, and Panama. In South America they comprised Bolivia, Brazil, British Guiana, Colombia, Ecuador,

Paraguay and Peru Foundation aid often involving minor financi
port extended to a few other countries helped them carry on studi
the disease and develop facilities for vaccine production This group
cluded the Belgian Congo France Great Britain India and the Union
South Africa Without the enlightened interest and participation of th
cooperating countries the enterprise could never have achieved what it d
Governments not only extended a cordial welcome to The Rockefell
Foundation but provided in substantial amounts both man power and func
for the common purpose

It is obvious that in an enterprise that spanned the world and was con
tinuously active for more than three decades a large number of individuals
must have been employed It would be interesting to know how many there
were and what were their several qualifications but unfortunately only the
records of Rockefeller Foundation personnel are available for analysis With
out exaggeration it may be said that the numbers of workers in all categories
contributed by the cooperating countries were overwhelmingly greater than
those furnished by the Foundation

Seventy six Rockefeller Foundation staff members were associated at one
time or another with the yellow fever program The majority 60 were doc
tors of medicine who had special competence in epidemiology pathology
public health administration and laboratory procedures The remainder
was made up of seven entomologists four bacteriologists two zoologists one
biochemist one physicist and one sanitary engineer

The directors of the International Health Division of The Rockefeller
Foundation Mr Wickliffe Rose Dr Frederick F Russell Dr Wilbur A
Sawyer and Dr George K Strode must also be mentioned since they carried
the ultimate responsibility for the work and served in sequence throughout
the entire period of operations The technical leadership of Dr Russell and
Dr Sawyer was particularly vital

The Yellow Fever Commission of The Rockefeller Foundation was or
anized in 1916 Except for a brief interruption occasioned by the entry of
e United States into the First World War it functioned until the close
1921 Active yellow fever operations began in 1918 and were continuous
om 1918 through 1949 a span of 32 years During this period the 76
ntists served a total of 465 man years Starting with two members in 1918
staff increased to a maximum of 27 in 1929–1930 and gradually fell to
in 1949 Of the 465 man-years 52 were spent in the International
lth Division s Yellow Fever Laboratory in New York

Life for the yellow fever worker, though generally lacking in the amenities and always strenuous, was seldom unrewarding. It was nevertheless extremely hazardous, at least until a reliable method of vaccination was discovered. No less than six of the scientists who participated in the program died of yellow fever (Figs. 72, 73, 74, 75, 76, and 77).

Though field operations were not initiated until 1918, The Rockefeller Foundation, through its Yellow Fever Commission, began to incur expendi-



Fig. 72. Dr. Howard B. Cross, 1888–1921



Fig. 73. Dr. Adrian Stokes, 1887–1927

tures two years earlier. Thus, for a period of 34 years, 1916 through 1949, varying sums were spent on behalf of the yellow fever program. The annual totals are shown in Table 32. These same figures are regrouped in Table 33 to show the total expenditures by regions and countries. To give completeness to the picture, the number of years of cooperation in each country is indicated. An attempt to separate expenditures chargeable to research in contrast to control was abandoned when it became evident that accuracy could not be achieved. Nevertheless, in view of the interest that centers about the subject, a rough estimate was made, which indicates that in all probability at least five million dollars, or 36 per cent, of the grand total of expenditures was employed in research.

Attention may be directed to the cost of The Rockefeller Foun[dation] staff which looms large as a total of more than four million dollars broken down to show the average cost (including salaries travel and expenses) of the 76 scientists who rendered 165 man-years of service average per man year was $9 051 35 If interest is focused on the rela[tion]ship of staff costs to those of the projects engaged upon it will be seen



Fig 74 Dr Hideyo Noguchi 1876–1928



Fig 75 Dr Paul A Lewis 1879–1929

30 45 per cent of the total expenditures are assignable to staff and 69 55 per cent to projects

It is evident that among the several cooperating countries expenditures varied greatly The size of these expenditures is roughly an index of two variables the significance of operations and their duration Brazil Nigeria Colombia and Uganda along with the Yellow Fever Laboratory of the International Health Division in New York not only were most successful in advancing knowledge of the disease and in perfecting methods of control but also were among the projects of longest duration Brazil is outstanding in that more than five million dollars were expended in that country during a period of 28 years

Yellow fever vaccine though developed in the New York lab[oratory]

of the International Health Division and subsequently manufactured there was likewise produced in large quantities by the Brazilian and Colombian laboratories. The vaccine production costs in the latter laboratories are inseparable from other costs, whereas records show that $166,757.21 was expended by the New York laboratories for this purpose. Since 28,101,120 doses were distributed, the cost per dose amounted to $0.0106. If allowance



Fig. 76. Dr. William A. Young, 1889–1929



Fig. 77. Dr. Theodore B. Hayne, 1898–1930

is made for certain overhead expenses and the salaries of the staff members who worked on vaccine production, the cost per dose increases to approximately $0.022. A list of the recipients, together with their respective quotas, is presented in Table 31. Without exception the vaccine was supplied free of cost to the 33 different agencies. The United States Army and Navy obtained 67 per cent of the total.

The investment of The Rockefeller Foundation in its yellow fever program was large, but when compared with the magnitude of the expenditures made by the cooperating countries its impressiveness undergoes considerable shrinkage. A complete statement of these expenditures cannot be presented, but an example or two will confirm the validity of the comparison. From 1930 to 1949 inclusive Brazil expended for the maintenance of its

TABLE 32

International Health Division of The Rockefeller Foundation
Yellow Fever Expenditures

1916–1949

1916	$ 40 395 84
191	3 580 30
1918	41 965 25
1919	75 229 74
1920	103 139 28
1921	120 731 33
1922	215 734 24
1923	144 066 06
1924	533 008 10
1925	513 380 97
1926	462 295 08
192	347 959 26
1928	238 043 26
1929	307 700 06
1930	346 550 58
1931	445 7 0 10
1932	484 249 58
1933	491 328 95
1934	386 84 57
1935	3 0 506 99
1936	297 466 45
1937	335 265 94
1938	346 468 59
1939	297 520 63
1940	279 382 18
1941	335 642 87
1942	364 213 14
1943	417 263 55
1944	257 633 12
1945	336 964 56
1946	200 164 80
1947	256 484 62
1948	130 347 86
1949	84 408 19
	$ 9 612 146 04
Staff members (76) Salaries and expenses	4 208 8 9 66
	$13 821 025 0

TABLE 33

INTERNATIONAL HEALTH DIVISION OF THE ROCKEFELLER FOUNDATION
YELLOW FEVER EXPENDITURES

1916-194[illegible]

Regions and countries	*Number of years of operation*	*Total expenditures in U. S. dollars*
Africa		
Uganda (Central and East Africa)	13	$ 23[illegible] 258 6[illegible]
Nigeria (West Africa)	20	953 522 8[illegible]
Union of South Africa	3	25 236 33
Europe		
France	[illegible]	13 038 1[illegible]
North and Central America		
Central America and West Indies	4	2 956 05
Cuba	2	1 123 3[illegible]
Guatemala	2	15 197 39
Mexico and Central America	8	387 447 09
Panama	8	[illegible] 209 00
El Salvador	4	6 300 71
United States of America		
Laboratory of the International Health Division, New York, Research	1[illegible]	950 241 9[illegible]
Training of Personnel and Miscellaneous Expenditures	12	165 236 00
Yellow Fever Vaccine Manufactured by the International Health Division Laboratory	[illegible]	46[illegible] 757 24
South America		
Bolivia	[illegible]	25 592 69
Brazil	28	5 099 609 35
British Guiana	8	15 208 58
Caribbean Littoral and Amazon Valley	2	10 305 38
Colombia	1[illegible]	616 989 30
Ecuador	13	127 655 25
Paraguay	2	13 514 95
Peru	15	166 896 72
Other Countries of South America including International Administration	[illegible]	53 943 18
General		
Surveys and Investigations in Any Region		
The Yellow Fever Commission	[illegible]	179 454 18
Surveys, immunologic	3	30 359 01
Total Project Costs		$ 9 612 146 04
Payments made by The Rockefeller Foundation to its [illegible] scientists in the form of salaries, annuities, travel, commutation, insurance, and allowances for tropical service		$ 4 208 879 66
Grand Total		$13 821 025 70

National Yellow Fever Service, which was devoted to the study and contr
of the disease 432 110 715 60 cruzeiros or $26,210 113 79 when converte
into United States currency at the average annual rate of exchange

TABLE 34

INTERNATIONAL HEALTH DIVISION OF THE ROCKEFELLER FOUNDATION
YELLOW FEVER VACCINE DISTRIBUTION BY THE YELLOW FEVER LABORATORY IN NEW YORK
July 1, 1940, to December 31 1947

Recipients		*Number of Doses Shipped*
Africa		
East and Central Africa	4 118 400	
West Africa	2 164 400	
South Africa Johannesburg	208 000	6 490 800
Canada		
Connaught Laboratories		65 200
Colombia		
Instituto de Estudios Especiales Carlos Finlay		1 008 700
France		
Pasteur Institute Paris		48 000
Great Britain		
Government	112 400	
Wellcome Laboratories	200 000	312 400
India		
Government		926 800
Peru		
National Yellow Fever Service		60 000
Portugal		
Government		20 120
Spain		
Government		15 200
Venezuela		
Government	120 000	
Standard Oil of New Jersey	10 200	130 200
United States of America		
Army and Navy	18 893 940	
Rockefeller Foundation Laboratories	23 240	
Special reserve for retitration (New York Laboratories)	85 560	19 002 740
Others		24 260
Total		28 104 420

her examples may be cited by presenting Table 35, which compares
xpenditures of seven countries under joint budgets with those of The
feller Foundation during a period from 1911 to 1919 inclusive Here
the expenditures of these countries exceeded those of The Rocke
oundation in the ratio of three to two

TABLE [illegible]

INTERNATIONAL HEALTH DIVISION OF THE ROCKEFELLER FOUNDATION'S
YELLOW FEVER EXPENDITURE

Cooperative Program

[illegible]

Country	*Government*	*Rockefeller Foundation*
Uganda (Central and East Africa)	$ 144 030 00	$ 184 8[illegible]0 04
Nigeria (West Africa)	14[illegible] [illegible]54 00	14[illegible] 38[illegible] 45
Brazil	6[illegible]2 454 00	433 4[illegible] [illegible] 24
British Guiana *	[illegible]58 554 00	[illegible] 413 [illegible]8
Colombia †	[illegible]56 [illegible]3 00	10 4[illegible]0 [illegible]4
Ecuador ‡	6 [illegible] [illegible]1 00	34 321 19
Peru	100 [illegible] [illegible] 00	[illegible] 18 5[illegible]
Totals	$1 556 [illegible] 6 00	$1 084 098 [illegible]

* 3 years of cooperation
† 5 years of cooperation
‡ 2 years of cooperation

The figures recorded in the preceding pages speak eloquently of the seriousness attached to yellow fever by countries in which it is a problem. They also confirm the assertion that an undertaking of this scope requires large scale financial support.

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